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(54) Title: HIV PHARMACCINES

(57) Abstract: The invention relates to a recombinant polypeptide comprising amino acid sequence derived from at least one of an HIV gag gene product; an HIV pol gene product; or an HIV nef gene product, said sequence being mutated with respect to the natural sequence of said gene product, and said sequence maintaining each of the naturally occurring CD8+ T cell epitopes of said gene product as defined in p17 and p24 (gag), amino acids 1-440 of RT (pol) and nef shown in Example 8. Furthermore the invention relates to nucleic acids encoding same, and viral vectors encoding same, and to their use in medicine and in immunisation and vaccination.

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HIV Pharmaccines

FIELD OF THE INVENTION

The invention relates to polypeptides comprising HIV antigens, in particular to polypeptides comprising mutated HIV sequences, said mutated sequences retaining their naturally occurring CD8+ epitopes. Furthermore, the invention relates to mutated HIV sequences into which extra T helper epitopes have been introduced.

BACKGROUND TO THE INVENTION

HIV is a pathogenic virus leading to debilitating and fatal immune deficiencies such as AIDS. Although there are certain therapies for conditions such as AIDS which can prolong life expectancy and increase quality of life for affected individuals, the disease is usually terminal.

Eliminating or controlling the virus in HIV infected patients is a problem.

Prevention of infection with HIV is a problem which has thus far been mostly countered by social solutions such as modification of sexual behaviour and/or greater take up of sterile practices for users of hypodermic needles.

Medical prevention of HIV infection remains a high priority area of research. There is a need for effective HIV vaccination strategies.

Even when immune responses are mounted against certain element(s) of an HIV particle, there are problems of immunodominance, viral escape and presentation of viral proteins which can lead to amelioration of the response. Clearly, it is extremely hazardous to render HIV particles for use in vaccines. Furthermore, use of proteins identical to natural proteins may itself be problematic if these proteins have undesirable effects. Furthermore, the proteins may themselves have

immunosuppressive qualities and/or may lack sufficient CD8+ T cell epitopes for a suitably strong or broad immune response to be provoked.

Viral escape by mutation of CD8+ T cell epitopes, especially immunodominant CD8+ T cell epitopes, is a significant problem in viral vaccination. Existing vaccines can exhibit poor recruitment of T helper cells and hence produce a narrow and/or weak immune response. Natural viral sequences, subject to evolutionary pressures in vivo, may possess relatively few immunologically significant epitopes. Furthermore, immunodominant effects can skew the clinical importance of certain CD8+ T cell epitopes and focus the response (if any) on these. Clearly, this serves to accentuate immunodominance problems, for example leading to sub selection of viral lines which mutate at this epitope, leading to potentially fatal viral escape (eg. see Barouch et al. 2002 Nature 415 p.335).

WO02/32943 (Nabel and Huang) disclose modifications of HIV ENV, GAG and POL to enhance immunogenicity for genetic immunization. In particular, they focus on modification of glycosylation of ENV, and on nucleic acid constructs encoding delta CFI HIV ENV. Furthermore, specific deletions of ENV cleavage site, fusogenic domain, and spacing of heptad repeats 1 and 2 are disclosed. Immunization with DNA plasmids encoding GAG alone or GAG-POL is described, the best results being alleged for the gag-pol plasmid immunisation. The bulk of WO02/32943 is concerned with the disclosure of specific plasmids listed in table 1 and the claims of WO02/32943. Also claimed are 'analogs' of various parts of these plasmids, and segments having at least 95% sequence identity thereto. To disrupt functions of Nef such as limitation of MHC class I and/or CD4 expression in WO02/32943, point mutations were introduced into the Nef gene from HIV-1 PV22. PV22 corresponds with the HXB2 reference sequence (see example 8) at the positions of mutations. Thus it can be seen that the mutations of WO02/32943 destroy naturally occurring epitopes. For example, amino acid substitutions in the Nef polypeptide of construct VRC4301 are: P69A, P72A, P75A, P78A, D174A and D175A. Each of the 6 mutations lies within a defined epitope with reference to Example 8, and therefore the naturally occurring epitopes have not been retained in this modified Nef gene. In constructs

VRC 4306, VRC 4309, VRC 4310, VRC 4311, VRC 4312 and similar construacts in WO02/32943, the reverse transcriptase mutation made (D771H) falls within the YMDD motif (shown in epitope map as RT 183-186) which is an area with a number of recorded epitopes. Thus these epitopes have not been retained in these prior art constructs. Furthermore, synthetic gag/pol/nef genes disclosed in WO02/32943 have been made by truncating fusions such as described for VRC 4312 and similar constructs. With reference to VRC 4312, the synthetic gag gene was ligated in frame with sequences encoding synthetic nef gene that 51 aa were deleted from 5'; 77 aa were deleted from 5' of pol polyprotein, and ligated with 3' of nef in which tag stop codon was deleted. These truncations of pol and nef result in loss of epitopes and so the naturally occurring epitopes have not been retained in terms of full length gag/pol/nef genes.

WO02/022080 (Merck and Co.) disclose modifications of HIV1-Gag, Pol and Nef. The principal modifications described are codon optimisation of the Gag, Pol and Nef genes. There is no mention of mutation of Gag polypeptide, only codon optimisation of the nucleic acid encoding it. Example 17 of WO02/022080 presents various mutations of the Pol protein; nine point mutations are presented, three of which are localised to each of the reverse transcriptase, RNAse and integrase regions of the protein. At least the D112A, D187A, D188A, D445A and D500A mutations of HIV-1 Pol are within human epitopes and therefore the naturally occurring epitopes have not been retained. At least the 'LLAA' mutations of FHV-1 and IRV-1 Nef (ie. L164A, L165A double mutant) are within human epitopes and therefore the naturally occurring epitopes have not been retained in this mutated Nef polypeptide.

WO03/025003 discloses various altered gag gene constructs, and also mention nef and pol gene constructs. The nef gene constructs appear to be truncated removing epitopes from the N-terminus of nef. The gag gene constructs appear not to be mutated.

The present invention seeks, *inter alia*, to overcome some of the problem(s) discussed above.

SUMMARY OF THE INVENTION

The present invention is based on the design of particularly effective presentation of HIV derived CD8+ T cell epitopes. In this way, the strongest and broadest immune response can be produced.

This is accomplished by altering the context of the polypeptide on which the epitopes are carried. In this way, the natural biological function(s) of the viral polypeptides can be destroyed, advantageously rendering them safe for vaccination use, whilst carefully preserving naturally occurring epitopes which prior art techniques are known to destroy.

In this and other aspects, supplementary (ie. non-naturally occurring) T helper epitopes are also introduced into the antigenic polypeptides, thereby advantageously strengthening and broadening the immune response.

Thus, in one aspect the invention provides a recombinant polypeptide comprising amino acid sequence derived from at least one of

(i) an HIV gag gene product;

(ii) an HIV pol gene product; or

(iii) an HIV nef gene product,

said sequence being mutated with respect to the natural sequence of said gene product, and said sequence maintaining substantially all of the naturally occurring CD8+ T cell epitopes of the corresponding part(s) of said gene product as defined in p17 and p24 (gag), amino acids 1-440 of RT (pol) and nef shown in Example 8.

In another aspect, the invention relates to a recombinant polypeptide as described above comprising amino acid sequence derived from at least two of (i), (ii) and (iii). When the polypeptide comprises amino acid sequence derived from only two of (i), (ii) and (iii), preferably they are (i) and (iii) ie. gag and nef.

In another aspect, the invention relates to a recombinant polypeptide as described above comprising amino acid sequence derived from (i) and (ii) and (iii).

Preferably each sequence derived from (i), (ii) and (iii) which the recombinant polypeptide comprises is mutated with respect to the natural sequence of said gene product. Preferably each said sequence maintains substantially all of the naturally occurring CD8+ T cell epitopes, preferably all said epitopes.

In another aspect, the invention relates to a recombinant polypeptide as described above wherein the amino acid sequences derived from (i) and/or (ii) and/or (iii) are arranged in the order (i) - (ii) - (iii) from the N terminus to the C terminus of the polypeptide.

In another aspect the invention relates to a recombinant polypeptide comprising amino acid sequence derived from

(i) an HIV gag gene product;

(ii) an HIV pol gene product; and

(iii) an HIV nef gene product,

said sequence being mutated with respect to the natural sequence of said gene product, and said sequence maintaining substantially all of the naturally occurring CD8+ T cell epitopes of said gene product as defined in p17 and p24 (gag), amino acids 1-440 of RT (pol) and nef shown in Example 8, said polypeptide comprising amino acid sequence having at least 75% identity to SEQ ID NO:9. Preferably said polypeptide comprises amino acid sequence having at least 95% identity to SEQ ID NO:9.

In another aspect, the sequence identity is interepitope sequence identity. This means that the epitopes correspond with 100% sequence identity to the epitopes in the natural or reference sequence, and the sequence identity quoted is for the remaining non-epitope regions of the sequence ie. the interepitope regions. Preferably this is calculated across all interepitope regions present. Preferably 'epitope' refers to the linear amino acid sequence to which the epitope has been mapped, preferably with reference to the epitopes mapped in Example 8.

In another aspect, the invention relates to a recombinant polypeptide as described above comprising SEQ ID NO:9, or a sequence having at least 95% identity thereto.

In another aspect, the invention relates to a recombinant polypeptide as described above further comprising an antibody recognition tag.

In another aspect, the invention relates to a recombinant polypeptide as described above wherein said tag is an HA tag comprising the sequence as shown in SEQ ID NO:8.

In another aspect, the invention relates to a recombinant polypeptide as described above further comprising a CD8+ T cell epitope tag.

In another aspect, the invention relates to a recombinant polypeptide as described above wherein said tag is a gp160 derived tag comprising the sequence as shown in SEQ ID NO:7.

In another aspect, the invention relates to a recombinant polypeptide as described above, said polypeptide comprising the sequence as shown in SEQ ID NO:1.

In another aspect, the invention relates to a recombinant polypeptide as described above, said polypeptide comprising amino acid sequence derived from an HIV nef gene product, said recombinant polypeptide sequence being mutated to disrupt the function of said nef sequence, said nef sequence further comprising one or more T helper epitopes which are not present in the naturally occurring nef gene.

In another aspect, the invention relates to a recombinant polypeptide as described above comprising one or more T helper epitopes which are not present in the naturally occurring nef sequence and are shown in Figure 3A.

In another aspect, the invention relates to a recombinant polypeptide as described above further comprising substantially all of the naturally occurring nef CD8+ T cell epitopes as defined in Example 8.

In another aspect, the invention relates to a recombinant polypeptide as described above further comprising substantially all of the naturally occurring nef T helper epitopes as defined in Example 8.

In another aspect, the invention relates to a recombinant polypeptide as described above wherein said polypeptide comprises sequence as shown in SEQ ID NO:6, or a sequence having at least 95% identity thereto.

In another aspect, the invention relates to recombinant polypeptide as described above, said polypeptide comprising amino acid sequence derived from an HIV pol gene product, said recombinant polypeptide sequence being mutated to disrupt the reverse transcriptase activity of the pol sequence, wherein substantially all of the CD8+ T cell epitopes of the naturally occurring pol sequence as defined in amino acids 1-440 of RT (pol) shown in Example 8 are retained in said recombinant polypeptide.

In another aspect, the invention relates to a recombinant polypeptide as described above, wherein the reverse transcriptase activity of said pol sequence is mutated by duplication of an internal sequence derived from the centre of the naturally occurring pol gene and exchange of the amino and carboxy terminal portions of said pol sequence.

In another aspect, the invention relates to a recombinant polypeptide as described above wherein said duplicated internal sequence comprises TPDKKHQKEPPF (SEQ ID NO:4).

In another aspect, the invention relates to a recombinant polypeptide as described above wherein said polypeptide comprises sequence as shown in SEQ ID NO:12 or a sequence having at least 95% identity thereto.

In another aspect, the invention relates to a recombinant polypeptide as described above, said polypeptide comprising amino acid sequence derived from an HIV gag gene product, said recombinant polypeptide sequence being mutated to disrupt processing of the gag gene product, and said gag sequence further comprising a disrupted myristoylation site, wherein substantially all of the CD8+ T cell epitopes of the naturally occurring gag sequence as defined in p17 and p24 (gag) shown in Example 8 are retained in said recombinant polypeptide.

In another aspect, the invention relates to a recombinant polypeptide as described above wherein the processing of gag is disrupted by exchanging the p17 and p24 domains and wherein the myristoylation site is disrupted by mutation of the second glycine to alanine.

In another aspect, the invention relates to a recombinant polypeptide as described above wherein said polypeptide comprises sequence as shown in SEQ ID NO:13 or a sequence having at least 95% identity thereto.

In another aspect, the invention relates to a recombinant polypeptide as described above wherein the HIV is a clade B HIV.

In another aspect, the invention relates to a recombinant nucleic acid encoding a polypeptide as described above.

In another aspect, the invention relates to a recombinant nucleic acid sequence comprising SEQ ID NO:11, or a sequence which differs only by silent mutations with respect to the genetic code, or a sequence having at least 95% identity thereto.

In another aspect, the invention relates to a viral vector encoding a polypeptide as described above.

In another aspect, the invention relates to a viral vector as described above wherein said vector is an MVA or MVA derived vector.

In another aspect, the invention relates to a viral vector as described above wherein said vector is a fowlpox or fowlpox derived vector.

In another aspect, the invention relates to a viral vector as described above wherein said vector is a FP9 fowlpox vector. Specific teachings with regard to this vector may be found in WO03/047617 which is incorporated herein by reference.

In another aspect, the invention relates to the use of a polypeptide as described above in medicine.

In another aspect, the invention relates to the use of polypeptide as described above in the preparation of a medicament for the treatment or prevention of HIV infection.

In another aspect, the invention relates to the use of polypeptide as described above in the preparation of a medicament for immunisation against HIV infection.

In another aspect, the invention relates to the use of a nucleic acid as described above in medicine.

In another aspect, the invention relates to the use of nucleic acid as described above in the preparation of a medicament for the treatment or prevention of HIV infection.

In another aspect, the invention relates to the use of nucleic acid as described above in the preparation of a medicament for immunisation against HIV infection.

In another aspect, the invention relates to a method of immunising a subject against HIV infection comprising administering to said subject a polypeptide or nucleic acid as described above.

In another aspect, the invention relates to the use of a polypeptide or nucleic acid as described above as a priming agent or as a boosting agent in a prime-boost immunisation regime. Prime boost immunisation is well known in the art, and specific teachings on this subject may be taken from WO98/056919, which is incorporated herein by reference.

In another aspect, the invention relates to the use of a polypeptide or nucleic acid as described herein in the induction of an immune response. Said immune response may be, for example, a cellular immune response, such as a CD8+ or CD4+ response, or a humoral (antibody) response. The invention moreover provides a method for eliciting an immune response in a subject comprising administering to said subject, which may be in need of such administration, a polypeptide, nucleic acid or vector as herein described.

In another aspect, the invention relates to a nucleic acid vector comprising a nucleic acid sequence as described above or encoding a polypeptide as described above.

In another aspect, the invention relates to an adenovirus vector comprising a nucleic acid sequence as described above or encoding a polypeptide as described above.

In a further aspect, the invention relates to a vector based on VSV (vesicular stomatitis virus), adeno-associated virus (AAV), alphavirus, Sendai virus or Herpes Simplex virus comprising a nucleic acid sequence as described above or encoding a polypeptide as described above.

In another aspect, the invention relates to a poxvirus vector comprising a nucleic acid sequence as described above or encoding a polypeptide as described above.

In another aspect, the invention relates to a plasmid selected from the group consisting of p29D.gpn, pOPK6.gpn and pSG2.gpn.

DETAILED DESCRIPTION OF THE INVENTION

DEFINITIONS

As used herein, the term "adenovirus" comprises the members of the Adenoviridae (adenovirus family). This family, in turn, comprises three genera: Mastadenovirus, Aviadenovirus and ATadenovirus. In particular, the invention contemplates the use of ovine adenovirus (an ATadenovirus).

A "CD8+ T cell epitope" is an amino acid sequence which is a peptide recognised by CD8+ T cells usually in conjunction with a class I major histocompatibility complex. In particular, reference to "all" CD8+ T cell epitopes, and/or "all known" CD8+ T cell epitopes, refers to currently known epitopes, as defined in Example 8 hereto, HIV Molecular Immunology 2002: Maps of CTL Epitope Locations Plotted by Protein; Theoretical Biology & Biophysics, Los Alamos National Laboratory, August 7, 2003. In accordance with the present invention, substantially all human CD8+ T cell epitopes are retained in the modified polypeptides; however, CD8+ T cell epitopes relevant in other mammalian species, such as murine CD8+ T cell epitopes, may be lost. CD8+ T cells are synonymous with CTLs (cytotoxic T-lymphocytes).

A "T helper epitope", likewise, is a peptide recognised by T helper cells usually in conjunction with a class II major histocompatibility complex; "all" and/or "all known" T helper cell epitopes are as defined in Example 8, HIV Molecular Immunology 2002: Maps of CTL Epitope Locations Plotted by Protein; Theoretical Biology & Biophysics, Los Alamos National Laboratory, August 7, 2003. In accordance with the present invention, substantially all human T helper cell epitopes are retained in the modified polypeptides; however, T helper epitopes relevant in other mammalian species, such as murine T helper epitopes, may be lost. A T helper cell is synonynous with a helper T cell.

"Substantially all" means at least 99%; preferably, it means at least 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86% or 85%. In an alternative

embodiment, 'substantially all' refers to all except one, all except two, all except three, all except four, all except five, all except six, all except seven, all except eight, all except nine, all except ten, all except eleven, all except twelve, all except thirteen, all except fourteen, all except fifteen.

An epitope is considered to be 'lost' or 'destroyed' or 'not retained' if the peptide sequence to which it is mapped, for example with reference to Example 8, is mutated. Preferably all epitopes are retained, with reference to those shown in Example 8.

A 'natural' sequence or 'reference' sequence as used herein refers to the source sequence from which the claimed polypeptide or nucleotide sequence(s) are designed or derived. Wherever possible this term should take its ordinary meaning ie. referring to the sequence of the corresponding gene(s) in a virus found in nature. However, it will be apparent to a person skilled in the art that there is not a single HIV virus found in nature, but rather there are many different clades and many different isolates or clones within those clades. Therefore, when a particular sequence according to the invention has been designed or derived from a particular clone or isolate of HIV, then the 'natural' or 'reference' sequence refers to that particular clone or isolate's own sequence. However, there are numerous consensus sequences known in the art which have been formed and indeed are sometimes updated by the comparison or 'pileup' of a number of different isolate or clonal sequences which may be divergent at one or more nucleotide or amino acid positions. In the situation where a construct according to the present invention has been designed or derived from such a consensus sequence, then this consensus sequence will be taken to be the natural or reference sequence.

Preferably all epitopes for the 'full length' g/p/n gene products as described herein are retained. Preferably all said epitopes are retained even when non-epitope containing regions of the gene product are truncated or deleted.

The natural or reference sequence should preferably be considered on a gene-by-gene basis. For example, a construct according to the present invention could comprise a gag gene, a nef gene and a pol gene. These genes will each have a natural or reference

sequence. It may be that the natural or reference sequences for each gene will be from the same source eg. the same overall consensus sequence, or it may be that the natural or reference sequence for each will be from a different source, eg. the gag and pol reference sequences may be from the 2001 clade B consensus sequence whilst the nef reference sequence may be from the 2000 clade B consensus sequence or vice versa. Reference sequences (both consensus and clonal) may be found in the Los Alamos Database as discussed herein (HIV Molecular Immunology 2002: Maps of CTL Epitope Locations Plotted by Protein; Theoretical Biology & Biophysics, Los Alamos National Laboratory, August 7, 2003). Specifically the 2001 clade B consensus sequence may be found at http://www.hiv.lanl.gov/content/hiv-db/ALIGN_02/ALIGN-INDEX.html and the 2000 clade B consensus sequence may be found at http://www.hiv.lanl.gov/content/hiv-db/ALIGN_01/ALIGN-INDEX.html.

"HIV" is Human Immunodeficiency Virus, a virus that causes immunodeficiency by attacking CD4+ cells in the body. The term "HIV", as used herein, includes any HIV, including all groups and subtypes (clades) of HIV-1 and HIV-2, for example HIV-1 M and HIV-1 O groups; the invention embraces each of the known clades; Clade B HIV-1 is preferred. Gag, pol and nef gene products are well known in the art and are as defined, for instance, in Example 8 hereto.

Guidance on the application of the invention to different clades may also be found in HIV Sequence Compendium 2002 Kuiken C, Foley B, Freed E, HahnB, Marx P, McCutchan F, Mellors J, Wolinsky S, and Korber B,editors. Published by the Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, LA-UR number 03-3564, incorporated herein by reference. In particular, consensus sequence data for HIV-1 clades A, B, C and D and a number of isolates can be found on pages 490 to 550; consensus sequence data for HIV-2 clades A, B, C and D and a number of isolates can be found one pages 554 to 578. Preferably the HIV is HIV-1 or HIV-2, most preferably HIV-1. Preferably the HIV is of clade A, B, C or D, preferably of clade B or D, preferably of clade B. An HIV sequence can be aligned to the HXB2 reference sequence (example 8), and therefore the g/p/n genes can be identified and corresponding epitopes delineated, no matter which clade is used. This can be done by

eye, but is preferably done using the Los Alamos database's "HXB2 Numbering Engine" for this purpose (http://www.hiv.lanl.gov/content/hiv-db/NUM-HXB2/HXB2.MAIN.html).

A "mutation", as referred to herein, encompasses any addition, deletion or substitution of amino acids in a polypeptide or nucleic acid. Mutations, in general, alter the amino acid sequence of the polypeptide in question such that it differs from a or the naturally occurring polypeptide sequence.

In the context of the present invention, mutations are often introduced in order to abrogate or ameliorate a known biological activity of the viral protein. Thus, preferably when a sequence is said to be mutated with respect to the natural sequence, this means mutated to reduce or preferably remove one or more biological activities of the viral polypeptide. Examples of specific mutations which result in the reduction or removal of particular viral biological activities are presented herein. Further examples of motifs or domains associated with viral functions are known in the art. However, in accordance with the present invention, it is important that when the mutations are made to reduce/remove the biological functions, the naturally occurring epitopes are preserved as explained herein.

A "recombinant" polypeptide, as referred to herein, is a peptide whose sequence differs from a or the naturally occurring equivalent polypeptide and which may be produced by genetic recombination technologies, including DNA synthesis and manipulation. Recombinant peptides also includes peptides which are not produced by recombinant means, but which have designed using recombinant DNA technology or, preferably, have a sequence identical to a peptide designed by such technology.

An "immune response" as referred to herein, is either a cellular (to include, but not limited to, CD4+ and CD8+) or humoral response to an antigenic sequence or a combination of both.

The gene names used herein to define segments of nucleic acid and/or polypeptide sequence such as 'gag', 'pol', 'nef' and the like preferably have their ordinary meaning. Most often the terms are used to describe the polypeptide sequence, or the corresponding nucleotide sequence encoding said polypeptide sequence.

A "protective immune response" as referred to herein, is an antigen specific immune response that provides a prophylactic and/or therapeutic benefit.

VIRAL ESCAPE

Mutations can occur in any epitope and lead to viral escape. By providing a greater number of epitopes it is more likely that some of the epitopes will remain unmutated. Furthermore, by advantageously providing extra T helper epitopes, the immune response can be broadened and/or strengthened according to the present invention.

Thus, in one embodiment, the present invention advantageously counters immunodominant effects which can affect conventional vaccines.

It is demonstrated that all of the identified nef human epitopes (both CD8+ T cell and T helper epitopes) in the HIV molecular immunology database are present in the GPN sequence of the present invention (SEQ ID NO: 1).

As noted above, WO02/32943 (Nabel and Huang) makes various disclosures in the field of HIV vaccines. As will be apparent from this specification, the present invention is distinct from disclosures made therein. In preferred embodiments, the present invention relates to materials comprising and/or encoding HIV derived proteins which expressly exclude any of those disclosed in WO02/32943, and preferably exclude any having 95% or greater identity to those disclosed in WO02/32943.

As noted above, WO02/022080 (Merck and Co.) makes various disclosures in the field of HIV vaccines. As will be apparent from this specification, the present invention is

distinct from disclosures made therein. In preferred embodiments, the present invention relates to materials comprising and/or encoding HIV derived proteins which expressly exclude any of those disclosed in WO02/022080, and preferably exclude any having 95% or greater identity to those disclosed in WO02/022080.

As noted above, WO03/025003 makes various disclosures in the field of HIV. As will be apparent from this specification, the present invention is distinct from disclosures made therein. In preferred embodiments, the present invention relates to materials comprising and/or encoding HIV derived proteins which expressly exclude any of those disclosed in WO03/025003, and preferably exclude any having 95% or greater identity to those disclosed in WO03/025003.

CD8+ T CELL EPITOPES

CD8+ T cell epitopes can be identified experimentally and can be predicted by analysis of the sequence of interest. Preferably these epitopes are predicted/recognised using the ProPred program (epitope prediction program, employing a matrix based prediction algorithm as disclosed in Sturniolo et al. Nat. Biotechnol. 17. 555-561(1999) and Singh and Raghava (2001) Bioinformatics,17(12), 1236-37, such as may be found at (http://www.imtech.res.in/raghava/propred/)).

It is an advantage of the present invention that naturally occurring epitopes are preserved in the polypeptide(s) of interest and in nucleic acids encoding them.

Addition or introduction of new CD8+ T cell epitopes may occur in the process of mutation and gene construction.

T HELPER EPITOPES

T helper epitopes can be identified experimentally and can be predicted by analysis of the sequence of interest. Preferably these epitopes are predicted/recognised using the ProPred program (epitope prediction program, employing a matrix based prediction

algorithm as disclosed in Sturniolo et al. Nat. Biotechnol. 17. 555-561(1999) and Singh and Raghava (2001) Bioinformatics,17(12), 1236-37, such as may be found at (http://www.imtech.res.in/raghava/propred/)).

Preferably, in the present invention naturally occurring T helper epitopes are preserved in the polypeptide(s) of interest and in nucleic acids encoding them.

Furthermore, the present invention advantageously provides novel T helper epitopes which have the beneficial effect of boosting and/or broadening the immune response to an antigen bearing said epitopes. In a preferred embodiment of the present invention, increased numbers of T helper epitopes are provided.

In another preferred embodiment of the invention, new T helper epitopes are created and/or introduced into the polypeptides of the present invention, thereby enhancing the immune response.

In some embodiments, the invention comprises a recombinant strain FP9 fowlpox and/or a recombinant modified vaccinia virus Ankara, (MVA), each expressing a novel fusion protein containing antigenic peptide sequences found in the translation products of the gag, pol and nef genes of human immunodeficiency virus type I, (HIV-1), preferably clade B.

The invention also relates to the use of such vectors in vaccination methods such as prime-boost (including single prime-multiple boost versions of prime-boost). Indeed, as discussed below, the use of either or both of these two recombinant viruses to boost a DNA plasmid-mediated prime has been shown to induce a strong immune response in mammals such as rodents and finds application in primates such as humans. The invention also relates to therapeutic immunotherapy for people infected with HIV-1, for example using the antigenic gene(s) of the present invention in combination with HAART, and/or a prophylactic immunotherapy for people at risk of infection with HIV-1.

Advantageously, vectors according to the invention employ appropriate codon usage to optimise protein expression from mammalian cells. Advantageously, human codon usage is employed.

In a preferred embodiment, therapeutic antigen(s) of the present invention comprise a fusion protein based on the products of the products of the HIV-1 (preferably clade-B) gag, pol and nef genes. Preferably said antigen(s) are delivered by one or both of the two recombinant pox viruses described herein.

In one aspect the invention provides a novel fusion protein containing antigenic peptide sequences derived from the translation products of the gag, pol and nef genes of human immunodeficiency virus type I, (HIV-1), preferably clade B.

A preferred amino acid sequence of the fusion protein is shown in Fig 1. A preferred nucleotide sequence encoding this amino acid sequence is shown in Figure 6. Naturally a person skilled in the art will appreciate that due to degeneracy of the genetic code, numerous possible nucleotide sequences are possible and this is only one preferred example of same.

The gene products are preferably in the order GAG-POL-NEF in the fusion protein. This is also the way they are arranged in the HIV genome. Other orders may be equally effective. It is well within the abilities of a person skilled in the art to alter the order to meet the needs of a particular application, or even merely to facilitate an easier construction and/or handling of the reagent(s).

In a preferred embodiment, the constructs of the present invention possess advantageous effects regarding immunodominance. Immunodominance has hindered prior art vaccines, especially with respect to gag epitope(s). This is discussed above. Three epitopes in GAG in a preferred GPN construct according to the present invention are studied in the example section and each mounted an immune response. It is further disclosed in a preferred embodiment how the immune response across the entire fusion protein is monitored (see below for more details).

It will be apparent that some of the GPN protein could be deleted, for example to reduce the size of the construct. Indeed, certain individual gene(s) are presented herein for individual application. Preferably the gpn constructs disclosed are not truncated. Without wishing to be bound by theory, it is suggested that a single protein may be less immunogenic if used alone as a smaller entity rather than used as disclosed within a gene. Thus, in a preferred embodiment, gpn genes disclosed herein are used without truncation/deletion. Preferably the g/p/n genes used are each full length.

It will be apparent to the skilled reader that the immunogenic components of the present invention are generally polypeptides. However, the invention relates both to these polypeptides and to nucleic acids encoding them, whether these take the form of DNA, plasmids, viral vectors or other such entities. Indeed, the actual mode of production of the antigenic polypeptides may be varied by a person skilled in the art according to the particular application to which the invention is being put. The polypeptides may be made *in vitro* or preferably *in vivo*, whether intra- or extra-cellularly. Specific modes of use are described herein.

GAG

The GAG protein has two functional domains, p17 matrix protein and p24 core capsid protein, both of which are essential and sufficient for assembly of HIV virus-like particles. In the gag gene(s) of the present invention, the p17 and p24 domains have advantageously been exchanged to disrupt processing of the GAG protein, in accordance with the International Aids Vaccination Initiative (WO01/47955). Other disruptional rearrangements are possible, such as scrambling as was applied to nef (see below), so long as the naturally occurring CD8+ T cell epitopes are preserved.

The myristoylation site of the p17 domain is advantageously mutated in order to prevent binding of the GAG protein to cellular membranes and, consequently, assembly and budding. This mutation is preferably by deletion or replacement of the second glycine residue in the domain with another amino acid, more preferably

replacement with an inert non-polar amino acid, most preferably by replacement of the second glycine residue in the domain with an alanine residue. This prevents GAG from functioning in binding to cellular membranes, in virus assembly and in budding.

Advantageously the immunogenicity of gag is not adversely altered in the recombinant gene(s) of the present invention.

Preferably 'full length' gag according to the present invention means comprising the full sequence of the p17 and p24 domains. Preferably full length gag comprises only the full sequence of the p17 and p24 domains.

POL

The pol gene encodes a number of proteins, including a protease, (p10), reverse transcriptase, (p51), RNase H, (p15) and an integrase, (p31).

The functional amino acid residues for reverse transcriptase activity centre on the YMDD motif (shown in epitope map as RT 183-186) which is an area with a number of recorded epitopes. Advantageously the reverse transcriptase is inactivated without loss of epitopes by domain swapping mutation as described in more detail below. Thus, in the present invention, preferably the reverse transcriptase activity present in the P51 subunit is disrupted. This is preferably done by rearrangement of the encoding sequence such that an altered P51 polypeptide is made with disrupted RT activity. Most preferably the sequence TPDKKHQKEPPF (which is present close to centre of the P51 polypeptide), is duplicated and the sequence encoding the amino- and carboxy-terminal parts of the P51 polypeptide exchanged about the point between the duplicated sequences.

This novel technique disrupts the active site in POL to ameliorate reverse transcriptase activity. Furthermore, this is advantageously accomplished according to the present invention while retaining the immunogenicity of the gene product. If the active site is mutated eg. by insertion, deletion or other known replacement type mutation, a T cell

epitope would be destroyed. According to the present invention such epitope(s) are retained.

Preferably 'full length' pol according to the present invention means comprising all of the p51 domain of pol (ie. aa 1-440) which is often known as Reverse Transcriptase (RT). In Example 8, RT and RNase are combined in the section named RT CTL Map; appropriate care should be taken when reading this Example. Sometimes this may be referred to as '1-440 of RT'. Sometimes aa 1-440 of pol is referred to as 'truncated pol' (p51) to indicate that other parts of the full viral pol ORF are omitted such as the RNAse unit. However, references to 'pol', 'truncated pol' (p51) and 'full length pol' preferably mean pol p51 amino acids 1-440 as used herein and as will be apparent from the context.

NEF

The nef gene encodes a multifunctional protein which enhances virus growth and mediates immune evasion. The NEF protein encoded by the gene in the current invention has been inactivated (ie. its function disrupted). In a preferred embodiment this inactivation is by dividing the coding region into eight subregions which were then reordered. Preferably these eight regions comprise 6 regions of 26 amino acid and two regions of 25 amino acids. A highly preferred scrambled NEF sequence is presented below (see Figure 2). Exact junctional boundaries in this sequence are easily identified by comparison to the native nef sequence (also presented below).

The objective of scrambling according to the present invention is to disrupt the functions of nef whilst preserving its T cell epitopes.

In scrambling nef according to the present invention, attention should be paid to disrupt the function of nef whilst creating a minimal number of surplus CD8+ T cell epitopes.

SEQ ID NO:10 is the native HIV nef sequence of the HIV clade B consensus sequence protein.

SEQ ID NO:11 is an exemplary nucleotide sequence coding for GPN. Naturally the person skilled in the art will appreciate that due to the degeneracy of the genetic code, many variant nucleotide sequences could equally code for the GPN of the present invention, especially those related to SEQ ID NO:11 and varying only by translationally silent differences in nucleotide sequence.

As used herein the term sequence 'derived from' an HIV gene product has its natural meaning in the art. Derived from simply indicates that it is based on the HIV sequence eg. as a starting point, whether this is *in silico* or actually experimental derivation eg. by cloning, PCR etc. For example the term would include predicted amino acid sequence from an HIV ORF, and is not limited to experimentally derived sequence. If a skilled person can recognise that the sequence originates from HIV, however much it has been rearranged or mutated, then it will be considered to be 'derived from' HIV. In a preferred embodiment, the sequence derived from another will possess a number of contiguous residues (amino acid or nucelotide) identical to the sequence from which is derived. Preferably there will be at least 5 such residues, preferably at least 8 such residues, preferably at least 10 such residues, preferably at least 14 such residues, preferably at least 18 such residues, preferably at least 20 such residues, preferably at least 22 such residues, preferably at least 25 such residues. For example, the gag, pol and nef sequences given herein are each derived from HIV.

The term 'mutated' has its usual meaning and includes deletion, insertion, point mutation, truncation, inversion as well as site directed mutation and scrambling as described herein.

It will be appreciated that the invention also embraces nucleic acid fragment(s) of the disclosed nucleotide sequences. Preferably nucleic acid fragments comprise at least 40 nucleotides, preferably at least 50 nucleotides, preferably at least 100 nucleotides, preferably at least 200 nucleotides, preferably at least 400 nucleotides, preferably at

least 600 nucleotides, preferably at least 800 nucleotides, preferably at least 1000 nucleotides, preferably at least 1500 nucleotides, preferably at least 2000 nucleotides, preferably at least 2500 nucleotides, preferably at least 3000 nucleotides, preferably at least 3400 nucleotides, preferably at least 3410 nucleotides.

It will be appreciated that the invention also relates to nucleic acids which differ from those presented only by virtue of the degeneracy of the genetic code. The open reading frame(s) of importance when judging variation connected to degeneracy of the genetic code will be those which encode polypeptide(s) of the present invention, particularly encoding core GPN (SEQ ID NO:9 and nucleic acids encoding it).

Unnatural nucleotide residues or analogues or derivatised moieties may feature in nucleic acids according to the present invention.

It will be appreciated that the invention also embraces polypeptide fragment(s) of the disclosed amino acid sequences. Preferably fragments are considered on a gene-by-gene basis. Preferably polypeptide fragments comprise at least 8 amino acids, preferably at least 9 amino acids, preferably at least 10 amino acids, preferably at least 12 amino acids, preferably at least 15 amino acids, preferably at least 20 amino acids, preferably at least 25 amino acids, preferably at least 26 amino acids, preferably at least 30 amino acids, preferably at least 40 amino acids, preferably at least 60 amino acids, preferably at least 80 amino acids, preferably at least 100 amino acids, preferably at least 150 amino acids, preferably at least 200 amino acids, preferably at least 300 amino acids, preferably at least 400 amino acids, preferably at least 600 amino acids, preferably at least 800 amino acids, preferably at least 1000 amino acids, preferably at least 1108 amino acids, preferably at least 1125 amino acids.

It will be appreciated that when considering fragments of the polypeptides and nucleic acids of the present invention, the same importance is attached to the preservation of epitopes. That is to say, if only a part of a gene (such as amino acids 1-440 of reverse transcriptase (pol)) are present in a construct according to the present invention, then it is required that the corresponding naturally occurring epitopes for that part of the gene

In a preferred embodiment, a sequence encoding a reporter CD8+ T cell epitope, RGPGRAFVTI (SEQ ID NO:7), recognized by murine CD8-positive T cells specific for the gp160 protein, may be incorporated into a recombinant gene of the present invention. This has the advantage of allowing monitoring of the induction of CD8+ T cells following immunisation with GPN-containing vaccines. Advantageously the presence of the epitope is not known to affect the function of the fusion gene. In a highly preferred embodiment, this reporter epitope is absent from construct(s)/recombinant gene(s) for primate vaccination, such as human vaccination.

In a preferred embodiment, an additional antibody tag, YPYDVPDYA (SEQ ID NO:8), recognized by antibodies specific for this part of the influenza virus haemagglutinin protein, is added to the gene of the present invention (or to the nucleic acid encoding it). Preferably this is added to the carboxyterminus of the protein to allow the detection of expression in antibody-based immunoassays, such as 'western blot assays'. In a highly preferred embodiment, this tag is incorporated into the carboxyterminus of a recombinant gag-pol-nef gene, such as in a recombinant vector comprising a recombinant gag-pol-nef gene. In a highly preferred embodiment, this antibody tag is absent from construct(s)/recombinant gene(s) for primate vaccination, such as human vaccination.

PHARMACEUTICAL COMPOSITIONS

The present invention also provides a pharmaceutical composition comprising administering a therapeutically effective amount of the agent of the present invention (such as a recombinant HIV gene such as the recombinant gpn gene as discussed herein) and a pharmaceutically acceptable carrier, diluent or excipients (including combinations thereof).

The pharmaceutical composition may comprise two components – wherein a first component comprises a nucleic acid vector and a second component which comprises a viral vector thereof. The first and second component may be delivered sequentially, simultaneously or together, and even by different administration routes.

The pharmaceutical compositions may be for human or animal usage in human and veterinary medicine and will typically comprise any one or more of a pharmaceutically acceptable diluent, carrier, or excipient. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R. Gennaro edit. 1985). The choice of pharmaceutical carrier, excipient or diluent can be selected with regard to the intended route of administration and standard pharmaceutical practice. The pharmaceutical compositions may comprise as - or in addition to - the carrier, excipient or diluent any suitable binder(s), lubricant(s), suspending agent(s), coating agent(s) or solubilising agent(s).

Preservatives, stabilizers, dyes and even flavouring agents may be provided in the pharmaceutical composition. Examples of preservatives include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. Antioxidants and suspending agents may be also used.

There may be different composition/formulation requirements dependent on the different delivery systems. By way of example, the pharmaceutical composition of the present invention may be formulated to be delivered using a mini-pump or by a mucosal route, for example, as a nasal spray or aerosol for inhalation or ingestible solution, or parenterally in which the composition is formulated by an injectable form, for delivery, by, for example, an intravenous, intramuscular or subcutaneous route. Alternatively, the formulation may be designed to be delivered by both routes.

Where the agent is to be delivered mucosally through the gastrointestinal mucosa, it should be able to remain stable during transit though the gastrointestinal tract; for example, it should be resistant to proteolytic degradation, stable at acid pH and resistant to the detergent effects of bile.

Where appropriate, the pharmaceutical compositions can be administered by inhalation, in the form of a suppository or pessary, topically in the form of a lotion,

solution, cream, ointment or dusting powder, by use of a skin patch, orally in the form of tablets containing excipients such as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs, solutions or suspensions containing flavouring or colouring agents, or they can be injected parenterally, for example intravenously, intramuscularly or subcutaneously. For parenteral administration, the compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the solution isotonic with blood. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner.

PHARMACEUTICAL COMBINATIONS

The agent of the present invention may be administered with one or more other pharmaceutically active substances. By way of example, the present invention covers the simultaneous, or sequential treatments with an agent according to the present invention and one or more steroids, analgesics, antivirals or other pharmaceutically active substance(s).

The invention also finds application in a therapeutic immunotherapy for people infected with HIV such as HIV-1. The invention may be used in combination with HAART, and/or in combination with a prophylactic immunotherapy for people at risk of infection with HIV such as HIV-1.

It will be understood that these regimes include the administration of the substances sequentially, simultaneously or together.

The present invention will now be described by way of example, which is not intended to limit the scope of the appended claims, in which reference will be made to the following figures:

Brief Description of the Figures

Figure 1 shows preferred gpn gene constructs. The sequence is continuous and has only been separated here for clarity. Bold indicates sequence variation between GPN sequence and the HIV Molecular Immunology sequence (NB. not all such sequence variations are shown).

Figure 2 shows scrambled and native nef gene sequences used for MHC Class II epitope comparisons.

Figure 3 shows T helper epitopes for scrambled nef (fig 3A) and native nef (fig 3B).

Figure 4 shows a map of recombination plasmid, p29D.gpn, for the construction of a recombinant fowlpox strain FP9 expressing gag-pol-nef.

Figure 5 shows a map of recombination plasmid, pOPK6.gpn, for the construction of a recombinant MVA expressing gag-pol-nef.

Figure 6 shows the sequence of GPN. The GPN sequence is shown in normal text. The upstream region is shown in italics. 5-prime ApaI and 3-prime AscI sites are underlined. Initiating ATG and terminating TGA are shown in bold.

Figure 7 shows a bar chart of an IFN-γ ELISpot as described in Example 3.

Figure 8 shows a bar chart of an IFN-γ ELISpot as described in Example 5.

Figure 9 shows overlapping 20mer peptides used in an IFN-γ ELISpot assay. Amino acid length shown in brackets.

Figure 10 shows a bar chart of GAG-specific responses in ex.4 group 4 animals.

Figure 11 shows a bar chart of POL-specific responses in ex.4 group 4 animals.

Figure 12 shows a bar chart of NEF-specific responses in ex.4 group 4 animals.

Figure 13 shows bar charts of IFN-γ responses.

Figure 14 shows a bar chart of IFN-γ responses elicited against selected peptides from gpn-sequence.

EXAMPLES

Example 1: Analysis of CD8+ T cell and T helper epitopes in the GPN sequence.

We used ProPred to compare T helper epitopes for native NEF versus scrambled NEF. This program predicts that there are more T helper epitopes in scrambled NEF

according to the present invention. This demonstrates that the scrambled NEF sequence is at least as potent in eliciting T helper and CD8+ T cell responses as native NEF.

CD8+ T cell Epitopes

CD8+ T cells can recognise and eliminate virally infected T cells and have been associated with control of viraemia in HIV infection in man and SIV infection in monkeys.

Comparison of the GPN sequence (Figure 1) with the HXB2 reference sequence published in the HIV molecular immunology database ("HIV Molecular Immunology 2002: Maps of CD8+ T cell Epitope Locations Plotted by Protein" Theoretical Biology and Biophysics, Los Alamos National Laboratory. August 7, 203; http://hiv-web.lanl.gov/content/immunology/maps/ctl/ctl.pdf) is performed.

This comparison reveals that all of the known murine and human CD8+ T cell epitope sequences identified for native gag (p17, p24), truncated-pol (p51) and nef are present in the GPN polypeptide sequence. There are single amino acid differences between the GPN sequence and the HXB2 reference sequence. Epitope sequences are indicated on the protein sequences of HXB2 and provide a relative location of the defined epitopes although they may vary relative to the protein sequence from which they were defined (refer to HIV Molecular Immunology Database 2002: Section II-A-2 – HIV Protein Epitope Maps, p56; http://hiv-web.lanl.gov/content/immunology/pdf/2002/immuno2002.pdf).

Therefore, shuffling and rearrangements within the GPN sequence have not altered *per se* the capacity of this molecule to induce CD8+ T cell responses in mice, monkeys or man against the native gag (p17, p24), truncated-pol (p51) and nef sequences present in HIV.

Scrambling and rearrangement of the native polypeptides constituting the GPN protein may have created new CD8+ T cell epitopes unique to this molecule. Since these

epitopes are unlikely to be present in native gag, pol or nef sequences within the HIV virus, they are therefore unlikely to be of biological relevance for a novel antigen for inclusion in a vaccine against HIV. Consequently, these epitopes have not been investigated further in this example.

T helper epitopes

T helper immune responses enhance the CD8+ T cell effector response in terms of magnitude and breadth and therefore may enhance the efficacy of the CD8+ T cell response against HIV infection.

Comparison of the GPN sequence with the HXB2 reference sequence published in the HIV molecular database ("HIV Molecular Immunology 2002: Maps of T helper Epitope Locations Plotted by Protein" Theoretical Biology and Biophysics, Los Alamos National Laboratory. August 7, 203; http://hiv-web.lanl.gov/content/immunology/maps/helper/helper.pdf) reveals that all of the human T helper epitope sequences identified for native gag (p17, p24), truncated-pol (p51) and nef are present in the GPN polypeptide sequence. Therefore, shuffling and rearrangements within the GPN sequence have not reduced the capacity *per se* of this molecule to induce T helper responses against the native gag (p17, p24), truncated-pol (p51) and nef sequences present in the clade B virus in humans.

Since T helper epitopes are usually longer than 15 amino acids, fusion of the gag, truncated-pol and nef sequences in GPN is likely to have advantageously created novel epitopes bridging the points at which these genes or parts of genes have been fused. Moreover, shuffling of the nef gene is likely to have created a number of novel epitopes bridging the shuffled sections of resulting polypeptide. Such novel T helper epitopes advantageously may elicit immune responses when GPN is administered as a vaccine, thereby enhancing the breadth and strength of the biologically-relevant CD8+ T cell responses elicited by the polypeptide(s) of the present invention relative to the native GAG, truncated-POL and NEF proteins.

To demonstrate that shuffling had created potential new T helper epitopes, the native NEF and scrambled NEF polypeptide sequences (Figure 2) are compared using the Propred MHC Class II epitope prediction program, employing a matrix based prediction algorithm as disclosed in Sturniolo et al. Nat. Biotechnol. 17. 555-561(1999) and Singh and Raghava (2001) Bioinformatics,17(12), 1236-37, such as may be found at (http://www.imtech.res.in/raghava/propred/).

Comparison of these molecules reveals substantial changes in the predicted human MHC class II restricted T helper epitopes, with a greater number of predicted epitopes in the scrambled NEF sequence than the native NEF sequence (Figures 3A and 3B).

Thus, scrambling of the nef sequence has resulted in the creation of additional T helper epitopes for enhancement of the CD8+ T cell response against this polypeptide according to the present invention. These advantageous novel T helper epitopes comprise those epitopes shown in Figure 3A and which are absent from Figure 3B.

Similar novel T helper epitopes will have been generated across the fusion points between the proteins comprising the GPN polypeptide. Comparison as described above may be used to define/investigate these further.

Example 2: Recombinant GPN gene in DNA, MVA and Fowlpox

A recombinant gag-pol-nef (gpn) gene is constructed for expression of a GPN fusion protein. This gene construct is used in nucleic acid carrier such as DNA carrier (eg. plasmid carrier), as well as in MVA and fowlpox vectors and materials for construction of same.

The use of recombinant virus vectors such as MVA and/or fowlpox vectors to boost a DNA plasmid-mediated prime has been shown to induce a strong immune response in rodents.

Essentially the same constructs also find application in primates such as humans.

In this example, the therapeutic antigen delivered by the recombinant pox viruses comprises a fusion protein based on the products of the products of the HIV-1 clade-B gag, pol and nef genes.

The recombinant gag-pol-nef gene was synthesized as a series of overlapping oligonucleotides, amplified by use of polymerase chain reaction cloned into the commercially available plasmid, pUC19, to make pUC19.gpn, (or 02-213), and the nucleotide sequence determined.

The gag-pol-nef gene is subcloned into three plasmid vectors to make

i) a plasmid expression plasmid, pSG2.gpn for use as a priming agent for *in vivo* use such as preclinical testing of gpn-containing recombinant poxvirus vectors;

ii) a recombination plasmid, pOPK6.gpn, for the construction of a recombinant MVA expressing gag-pol-nef;

iii) a recombination plasmid, p29D.gpn, for the construction of a recombinant fowlpox strain FP9 expressing gag-pol-nef.

The expression plasmid, pSG2.Mel3, (Palmowski, MJ. et al 2002 J Immunol 168 p4391-4398) contains an expression cassette based on the human cytomegalovirus immediate-early promoter and intron combined with the bovine growth hormone gene polyadenylation signal to express a synthetic epitope string containing melanoma antigens. The expression plasmid pSG2.gpn is made by digesting pUC19.gpn with ApaI and AscI, blunt-ending the insert, and subcloning into blunted PstI-digested pSG2Mel3.

The MVA recombination plasmid, pOPK6 is made as follows. The plasmid is based on the commercially available cloning plasmid, pSP72, (Promega Inc.); the standard multiple cloning site being replaced with a synthetic linker containing unique restriction sites and the vaccinia virus P7.5 late-early promoter and late P11 promoter arranged in a head-to-head orientation. The linker also has the first twenty nucleotides

of the *Escherichia coli* LacZ, (beta-galactosidase), gene as found in the vaccinia virus recombination plasmid pSC11, (Chakrabarti et al 1985 Mol Cell Biol 5 p3403-3409). The synthetic linker is synthesized as a series of overlapping oligonucleotides, amplified by PCR, and cloned into the commercial cloning plasmid, pcDNA3.1, to make pLink, (or 02-363). The sequence of the 300 base pair linker is given below:

agatctttaattaatgcctaggcaattgagacggcgcgcccgggcactagtaagggcccgtgcaataaattagaatatattttc
tacttttaccagaaattaattgtacaatttattatttatgggtgaaaaacttactataaaaagcgggtgggtttggaattagtggtac
catgcatcttagaatatatgtatgtaaaaatatagtagaatttcattttgttttttctatgctataaatgaattcctcaagggatccgt
ctcctgcaggcatgctaagctagcggccggccctcgag

Part of the LacZ gene from pSC11 is subcloned into pLINK to make pLinkLacZ. The linker and LacZ gene were then subcloned into pSP72 as a XhoI-BglII fragment such that a complete beta-galactosidase-encoding open reading frame is formed 3-prime to the P11 late promoter to make pSP72LinkLacZ.

MVA flanking regions representing 1500 base pairs 5-prime and 3-prime to the EcoRI site in the MVA thymidine kinase (tk) gene were isolated from MVA by PCR using the following primer-pairs:

Left-hand, (5-prime) tk flank

Forward primer CAATTACAGATTTCTCCGTGATAGGT

Reverse primer CGTGCATGCGGCCGCAACAATGTCTGGAAAGAACTG

Right-hand, (3-prime) tk flank

Forward primer CAGGAATTCGCGGCCGCTGTGAGCGTATGGCAAACG

Reverse primer TCATTTGCACTTTCTGGTTCGTA

The 1500bp PCR products are cloned into the commercial cloning vector pTOPO-TA, (Invitrogen) and sequenced.

The right-hand flank is subcloned into pSP72LinkLacZ as an EcoRI-MfeI fragment to make p72LinkLacZR. The left-hand flank insert is then subcloned into p72LinkLacZR as an NheI-SphI fragment to make pOPK6.

Subsequent sequence analysis of this plasmid identified an additional 174 base pairs of sequence in the pOPK6 backbone plasmid introduced from the pTOPO-TA vector with the left-hand flank due to non-specific cleavage by the NheI enzyme.

The gag-pol-nef open reading frame was subcloned from pUC19.gpn into pOPK6 as an ApaI-AscI fragment to make pOPK6.gpn and the gpn insert sequenced to confirm no alterations had occurred during the subcloning steps.

This plasmid functions as a vehicle for introducing the gpn open reading frame, under control of the P7.5 late-early promoter, into the tk locus of MVA.

FP9 vector

The FP9 strain fowlpox recombination plasmid, pEFL29, was obtained from Dr Mike Skinner at the Institute for Animal Health, Compton, UK as published by Qingzhong, Y. et al 1994, Vaccine 12(6) p569-573.

The gpn open reading frame was subcloned from pUC19.gpn as a blunted KpnI-SacI fragment into the SmaI site of pEFL29 to make pEFL29.gpn. Sequencing of this plasmid showed that LacY and part of LacA had been introduced with the LacZ marker gene during the construction of pEFL29. Consequently, a linker was synthesized that allowed the deletion of all of the LacY and most of the remaining

LacA open reading frames by means of a BsrGI-BlpI digest of pEFL29 to make p29Delta. The sequence of the linker is given below.

TGAGCGCCGGTAGATACCATTATCAGCTGGTGTGGTGTCAGAAGTAATGTA
C

The gpn expression cassette was then subcloned from pEFL29.gpn as an AatII-SphI fragment into AatII-SphI cut p29Delta to make p29D.gpn. The gpn insert was then sequenced to confirm no alterations had occurred during the subcloning steps.

The recombination plasmids, pOPK6.gpn and p29D.gpn are used to introduce the P7.5-gpn expression cassette into MVA and FP9, respectively.

The recombinant poxviruses were made by homologous recombination of the virus genome with the relevant recombination plasmid in chicken embryo fibroblast, (CEF), cultures using standard molecular biology techniques as described in Current Protocols in Molecular Biology, Ed. F.M. Ausubel, John Wiley & Sons; or according to suppliers' or manufacturers' instructions.

MVA.gpn is made by infecting confluent cultures of CEF cells with a non-recombinant MVA, (stock 575FHE-K), derived from MVA passage number 575, (Mayr *et al.*, (1978) Zentralbl Bakteriol [b]. 167(5-6):375-90) at a multiplicity of infection of 0.1 for one hour. The infected cells are then transfected with between 0.5 and 2.0µg pOPK6.gpn plasmid DNA mixed with lipofectin (Invitrogen), according to the manufacturer's protocol. The cultures are incubated for three days before harvesting. The harvested cells are freeze-thawed three times and titrated as ten-fold dilutions and cultured under CMC-containing medium for three days. The medium is removed and replaced with CMC-medium containing X-Gal and the cultures incubated in this medium for 20 hours.

Recombinant viruses are identified by a blue plaque phenotype and picked with a pipette into cryotubes containing 100µl medium. The picked viruses are plaque

purified five more times to ensure purity. Purity from non-recombinant parental virus was confirmed by the absence of white plaque phenotype and the absence of a WT PCR product when the virus DNA is screened using appropriate primers.

Purity PCR screening was performed on virus DNA purified from 2ml cultures infected with fifth or sixth round clones of MVA.gpn.

The virus DNA is purified using a Qiagen Blood and Cell Culture DNA min-kit (Qiagen GmbH, Max Volmar str. 4, 40724 Hilden, Germany) according to the manufacturer's protocol. A PCR reaction was performed using Klentaq proof reading polymerase (Sigma) using appropriate incubation conditions.

Parental virus was screened for using the following primers to give a band of 471 base pairs in the wild-type, but nothing in MVA:

TKU CAATTACAGATTTCTCCGTGATAGGT

TKL TCATTTGCACTTTCTGGTTCGTA

MVA.gpn was screened for using primer TKL and the following primer to give a band of approximately 2000 base pairs from the MVA.gpn construct:

HIV-U ATACCCCCGTGTTCGCCATTAAGA

Expression of the GPN protein from MVA.gpn is confirmed by immunoblotting as described below.

CEF cells are infected with MVA.gpn or MVA.LacZ control virus at a multiplicity of infection of 1. The culture is incubated for 3 days before harvesting. The cells are heated to 100 degrees C in SDS sample buffer and electrophoresed through a 4-20% acrylamide gel and electroblotted onto a nitrocellulose membrane. The transferred protein is then probed with either an antibody specific for the haemagglutinin tag

(Abcam), or one specific for HIV GAG P24, (Dako) and visualized using a suitable peroxidase-labelled secondary antibody and a chromogen. The blot assay indicates that the cells infected with MVA.gpn contain a protein of approximately 134kDa containing P24 and HA epitopes that was absent in the cells infected with MVA.LacZ. The predicted molecular weight of the GPN protein is 128.5 kDa, which is in close agreement with the specific band produced in the MVA.gpn-infected cells.

Expression of the GPN protein from FP9.gpn is confirmed by immunoblotting. CEF cells are infected with FP9.gpn or FP9.LacZ, ('FP9.29D'), control virus at a multiplicity of infection of 1. The culture is incubated for 5 days before harvesting. The cells are heated to 100 degrees C in SDS sample buffer and electrophoresed through a 4-20% acrylamide gel and electroblotted onto a nitrocellulose membrane. The transferred protein is then probed with either an antibody specific for the haemagglutinin tag (Abcam), or one specific for HIV GAG P24, (Dako) and visualized using a suitable alkaline phosphatase-labelled secondary antibody and a chromogen. The blot assay indicated that the cells infected with FP9.gpn contained a protein of approximately 134kDa containing P24 and HA epitopes that is absent in the cells infected with FP9.29D. The predicted molecular weight of the GPN protein is 128.5 kDa, which is in close agreement with the specific band produced in the FP9.gpn-infected cells.

The immunogenicity of the MVA.gpn and FP9.gpn viruses was tested in BALB/c mice using a 'prime-boost' protocol. The mice were divided into groups of four animals. The following test and control groups were established:

pSG2.gpn (50μ) prime followed 14 days later by a boost $1x10^6$ p.f.u. of MVA.gpn.

pSG2.gpn (50μ) prime followed 14 days later by a boost $1x10^6$ p.f.u. of FP9.gpn.

$1x10^6$ p.f.u. of MVA.gpn alone.

$1x10^6$ p.f.u. of FP9.gpn alone.

7 days after virus infection the peripheral blood lymphocytes were harvested and the number of GPN-specific interferon gamma-secreting lymphocytes determined in an ELISpot assay using the HIV following T cell epitopes (see also example 3):

H2-D CD8 epitopes:	AMQMLKETI
	TTSTLQEQ
Gp160 H2-D CD8 epitope:	RGPGRAFVTI
H2-D CD4 epitope:	NPPIPVGEIYKRWIILGLNK

Both MVA.gpn and FP9.gpn are shown to induce strong CD8+ T cell responses in the mice.

The prime-boost protocol is shown to give greater response than that seen in the groups receiving a single injection with virus.

Example 3: Immunisation with FP9.gpn and MVA.gpn elicits antigen specific CD8+ T cell responses

In this example it is demonstrated that FP9.gpn and MVA.gpn elicit enhanced antigen-specific CD8+ T cell responses when administered alone or in a prime-boost immunisation regime with pSG2.gpn. In this example, the demonstration is presented in mice.

Female BALB/c mice (6 – 8 weeks old) are immunised with 50μg of pSG2.gpn by intramuscular (im.) injection and boosted with 1 X 10^6 PFU FP9.gpn or 1 X 10^5 MVA.gpn by intravenous injection (iv.) two weeks later. A further group of age matched naïve female BALB/c mice are immunised iv. with 1 X 10^6 PFU FP9.gpn or 1 X 10^5 MVA.gpn at the same time as the booster immunisation.

Fourteen days after the booster immunisation, all mice are sacrificed by cervical dislocation and the T cell response elicited against three H-2^d restricted CD8+ epitopes

from the GPN polypeptide (Table A: AMQ, TTS, RGP) is determined by IFN-γ ELISpot assay as described below.

Murine IFNγ ELISpot protocol:

Materials:

IFN-γ ELISpot ALP Kit Mabtech 3321-2A

600µg anti-IFN-γ purified Mab AN18

50µg anti-IFN-γ biotinylated Mab R46A2

50µl Streptavidin-Alkaline Phosphatase

Complete α-MEM medium

500ml MEM α-modification Sigma M-4526

50ml FCS [10%] Sigma F-2442

5ml pen/strep [100U penicillin 100µg strep] Sigma P-0781

10ml L-glutamine [4mM] Sigma G-7513

500µl 2-Mercaptoethanol [50µm] Gibco BRL 31350-010

ACK buffer

8.29g NH_4Cl [0.15M] (Sigma A-4514)

1g $KHCO_3$[1mM] (Sigma P-9144)

37.2mg Na_2EDTA (Sigma ED2SS)

800ml milli-Q water

Adjust pH to 7.2-7.4 with HCl (Sigma S-7653)

Make up to 1000 ml with water and autoclave

Colour Development Buffer:

BioRad AP Conjugate Substrate kit (170-6432).

For one plate:

5ml deionised water

200µl of 25x buffer

50µl reagent A

50µl reagent B

Mix well and use immediately

Protocol

1. **Preparation of Plates:**

1.1. **Coating plates:** coat MAIP multiscreen plates (Millipore MAIPS4510) with rat anti-mouse IFNγ (Mab AN18) antibody. Dilute to 10µg/ml in Phosphate Buffered Saline (PBS; Sigma P-3813) and add 50µl per well to MAIP plates. Incubate overnight at 4°C in a humidified chamber

1.2. **Blocking plates:** Flick off coating antibody and wash plates once with 150 ul of sterile PBS (Sigma P-3813) per well using a multi-channel pipette. Flick off the PBS, add 100ul complete α-MEM medium per well, and incubate at room temperature for 1+ hour. It is important to keep the plates sterile at this stage.

2. **Splenocyte preparation:**

2.1. Crush individual spleens in 2 ml of PBS with the plunger of a 10 ml syringe in a 70 µm cell strainer (Falcon 352350) contained in a petri dish, add 5 ml of PBS, suspend splenocytes by pipetting, and transfer into a 50 ml tube. Rinse cell strainer and dish with a further 10 ml of PBS and add to the 50 ml tube. Centrifuge at 1500 rpm for 5 min.

2.2. Remove supernatant, re-suspend cells by tapping tube and add 5ml ACK buffer and mix by inversion. Incubate at room temperature for no longer than 5 minutes. Add 25 ml PBS, mix by inversion and centrifuge at 400 X g for 5 min.

2.3.Remove supernatant re-suspend pellet by tapping the tube, add 10ml PBS and vortex. Count using an improved Neubauer haemacytometer by diluting 1:10 in 0.4% trypan blue solution (Sigma T-8154). Aliquot amount needed for the Elispot and centrifuge at 1500 rpm for 5 min, resuspend by vortexing in an appropriate volume of complete Alpha MEM medium to give a concentration of 10 million cells/ml.

3. **Plate setup:**

50µl 50µl 50µl

← → → → ← → → → ← → → →

1	2	3	4	5	6	7	8	9	10	11	12
	150				150				150		
	150				150				150		
	150				150				150		
	150				150				150		
	150				150				150		
	150				150				150		
	150				150				150		
	150				150				150		
C				C				C			

Note: Plate layout should be varied according to needs. This layout is convenient for this example.

3.1.Flick blocking media from plate and add 50µl of complete alpha MEM medium to columns 3, 4, 7, 8, 11&12.

3.2.Add 150µl of splenocytes to columns 2, 6 and 10 in duplicate. (Up to 12 samples per plate)

3.3.Take 50µl of splenocytes from columns 2, 6 and 10 and transfer to columns 1, 5 and 9 respectively: these are the negative control wells.

3.4.Serially dilute each sample by taking 50µl from columns 2, 6 and 10, to columns 3, 7 and 11, mix well and transfer 50µl to 4, 9 and 12. Discard 50µl after mixing final columns in dilution.

3.5.Add test peptide and control peptide to twice the desired final concentration to naïve splenocytes at 10 million/ml in complete α-MEM medium. Add 50µl of control peptide and targeT cells to columns 1, 5 and 9.Add 50µl test peptide and targeT cells to remaining columns.

3.6.Incubate plates at 37°C for 18-20 hours.

4. **Developing the Assay**

4.1.Wash plates twice with PBS containing 0.05% Tween 20 (Sigma P1379), once with distilled water and twice with PBST.

4.2.Add 50µl/well of biotinylated rat anti-mouse interferon-gamma diluted to 1µg/ml in PBS. Incubate for 2 hours at room temperature.

4.3.Wash plates four times with PBST, then add 50 µl Streptavidin Alkaline Phosphatase (Mabtech) diluted to 1µg/ml in PBS. Incubate at room temperature for 1 hour.

4.4.Wash plates four times with PBST, add 50µl/well of colour development buffer Incubate at room temperature until spots develop (approx. 10 min). Wash plates well with tap water, peel off plastic bottom and leave to dry overnight on paper towels.

Calculation

Results are calculated as the number of epitope-specific IFNγ spot forming cells/million splenocytes (sfc/million). Differences between groups are determined by one-way ANOVA and a post hoc Tukey-Karamer multiple comparison test on $\log_{10}$ transformed data using GraphPad Instat version 3.05.

Results

The demonstration that immunisation with FP9.gpn and MVA.gpn alone or in prime-boost regimes with pSG2.gpn elicits antigen specific CD8+ T cell responses in mice is illustrated in figure 7. Female BALB/c mice were immunised im. with pSG2.gpn (DNA) or sham immunised with PBS (-) and boosted 14 days later with FP9.gpn (FP9) or MVA.gpn (MVA) as described above. The CD8+ T cell response was determined in splenocytes 14 days after the booster immunisation using the IFN-γ ELISpot assay. Columns represent the mean IFN-γ spot forming cells/million splenocytes ± standard deviation for 4 mice per group elicited by CD8+ reactive epitopes AMQ, TTS and RGP (see Table A).

Table A: GPN epitopes used in this study
([1]ND = not determined)

Antigen	**Abbr.**	**Epitope**	**CD4/CD8 reactive**	**MHC restriction**
HIV-1 env	RGP	RGPGRAFVTI	CD8	D^d
HIV-1 gag	AMQ	AMQMLKETI	CD8	K^d
HIV-1 gag	TTS	TTSTLQEQ	CD8	$H\text{-}2^d$
HIV-1 gag	NPP	NPPIPVGEIYKRWIILG LNK	CD4	$H\text{-}2^d$

Single or prime-boost immunisation with either FP9.gpn or MVA.gpn elicited a significantly ($P<0.01$) enhanced antigen-specific T cell response against each of the CD8+ T cell epitopes when compared to sham- immunised controls (Figure 7).

In addition, priming with pSG2.gpn and boosting with FP9.gpn or MVA.gpn elicited significantly ($P<0.01$) enhanced responses against each CD8+ T cell epitope when compared to single immunisations with each virus (Figure 7).

It should be noted that the virus titres were not identical in this experiment. Thus, the relative immune potency of the viruses has not been compared. Naturally it is straightforward to assess this by performing the example with identical viral titres.

Thus it is demonstrated that specific CD8+ T cell responses are elicited against the recombinant HIV genes according to the present invention such as the gpn gene by immunisation with either FP9.gpn or MVA.gpn, indicating that these constructs are both potent in eliciting an immune response against the GPN polypeptide.

Moreover, potent immune responses are directed at epitopes lying in the N- and C-terminal regions of the GPN polypeptide, indicating that the whole polypeptide is expressed, processed and presented after delivery with either FP9.gpn or MVA.gpn.

Both viruses elicit significantly higher immune responses against each epitope when administered to animals that have been primed with pSG2.gpn, indicating that both can act as boosting agents in prime boost immunisation regimes.

Example 4: Immunogenicity of FP9.gpn and MVA.gpn in primates

The immunogenicity of the gpn immunogens is demonstrated *in vivo* in primates as follows.

The immunogenicity of the GPN polypeptide consisting of the gag, pol and nef proteins of clade B HIV-1 is tested in non-human primates (Macaca mulatta).

The polyprotein (human codon usage) is expressed in recombinant MVA, fowlpoxvirus FP9, adeno virus and a DNA vaccine vector. The polyprotein expressing

constructs are administered in a heterologous immunisation regimen in order to induce high levels of antigen-specific CD8+ and CD4+ T cells.

Table B shows the study design of the **immunogenicity studies.**

Table B

Group No n=5	**Prime 1**	**Prime 2**	**Boost 1**	**Boost 2**
	Day 0	**Day 28**	**Day 56**	**Day 84**
1	FP9.gpn	FP9.gpn	MVA.gpn	MVA.gpn
2	MVA.gpn	MVA.gpn	FP9.gpn	FP9.gpn
3	-	FP9.gpn	MVA.gpn	-
4	pDNA+IL2-Ig/fc	MVA.gpn	FP9.gpn	Adeno.gpn

The design of the macaque study of this example is shown in Table B.

Blood samples are taken pre-immunisation and 7 days after each immunisation and four weeks after the last immunisation. PBMCs were cryopreserved and tested in ELISpot and intracellular cytokine assays using pools of overlapping peptides.

Cellular immune responses are tested during and at the end of the study. The frequency of IFN-γ-secreting T cells is tested by IFN-γ ELISpot assays (see Figure 9 for overlapping peptides used).

In an additional group, for comparitive purposes, DNA vaccines adjuvanted with IL-2/Ig fusion proteins (Barouch, D. H., A. Craiu, et al. (2000) Proc Natl Acad Sci U S A 97(8): 4192-7) and recombinant adenovirus are tested. For study design see Table B.

There are several parameters that are presented in this study:

(a) Order of FP9 and MVA. The most effective combinations of poxvirus vector primes and boosts are demonstrated. This particularly focuses on groups 1 and 2 of the study design (see Table B).

(b) Frequency of primes and boosts. The effectiveness (in terms of immunogenicity) of priming with a single poxvirus immunisation and boosting with a single heterologous poxvirus vector boost is demonstrated. This particularly focuses on animals in group 3 which will be immunised as outlined.

Read-out: The key objective of this vaccination strategy is to induce cellular immune responses. Therefore the following assays are used to monitor cellular immune responses: IFN-γ ELISpot using overlapping peptides (20mers overlapping by 10 amino acids as shown in Fig 9), intracellular cytokine staining for CD8+ and CD4+ T cells. In ELISpot assays CD4/8 depletion experiments will confirm IFN-γ secretion in response to peptides by CD4+ and/or CD8+ T cells.

Data for group 4 is presented below. The data shows immunogenicity of HIV immunotherapeutics in rhesus macaques using novel prime-boost immunisation regimens.

Table 1 Immunisation and sampling schedule

Group	d -28	d 0	d 2	d 7	d 28	d 35	d 56	d 63
4	Bleed	Bleed; i.m. inj. of 5 mg pfu pSG2. gpn	i.m. inj. of 5 mg pfu pS-IL-2fc	Bleed	Bleed; i.d. inj. of $5x10^8$ pfu of FP9. gpn	Bleed	Bleed; i.d. inj. of $5x10^8$ pfu of MVA. gpn	Bleed

Results

The GPN insert is immunogenic and induces interferon-gamma secreting T cells following viral vector immunisation in macaque monkeys.

In group 4 gpn-specific T cell responses are detected in 4/5 animals seven days after the FP9.boost. The responses are maintained following MVA boost.

This data shows that the gpn fusion protein is immunogenic in primates when it is delivered using recombinant vectors.

GAG, POL and NEF of gpn are recognised in immunised macaque monkeys.

Figures 10, 11 and 12 show responses of individual animals to different parts of the gpn protein indicating that T cell epitopes from the proteins gag, pol and nef are recognised in the same responding animal. For example animal N93 in Group 4 shows T cell responses recognising the GAG, POL and NEF portion of the GPN protein indicating that cell responses can be induced using GPN. Furthermore, without wishing to be bound by theory, the breadth of the immune response to the diverse elements of the triple GPN polypeptide indicates an advantageous feature of the invention in avoiding immunodominance effects where a single epitope can dominate the immune response to a polypeptide at the cost of responses to other epitopes present on the polypeptide. By contrast, the polypeptide according to the present invention of this example advantageously demonstrates a broad and balanced response.

Primate Immunogenicity Single Administration Experiments

The immunogenicity of the GPN polypeptide (SEQ ID No. 1) was tested in 10 rhesus macaques (Macaca mulatta), labelled A-J, in single administration ("single shot") experiments. In this example the polypeptide was delivered via either the MVA or FP9 vector.

ELISpot assays were carried out as described above. Responses to 119 overlapping ~20mer peptides (as described in Example 7) were measured and then summed to give a total response.

Responses were measured prior to injection and 28 days post injection. Responses are shown below. GPN elicited an immune response in all animals studied.

		Day 0 Bleed then i.d. $5x10^8$ pfu MVA.gpn	**Day 28 Bleed**
		Summed ELISpot response SFC/million PBMC	
Animal	A	13.35	28.33
	B	6.70	203.33
	C	23.40	133.33
	D	6.65	20.00
	E	0.00	320.00

		Day 0 Bleed then i.d. $5x10^8$ pfu FP9.gpn	**Day 28 Bleed**
		Summed ELISpot response SFC/million PBMC	
Animal	F	0	9.90
	G	28.35	48.33
	H	3.35	31.67
	I	0	98.33
	J	0	345.00

The results indicate that the GPN polypeptide is immunogenic and induces interferon-gamma secreting T cells following viral vector immunisation in primates such as macaque monkeys.

Thus the effectiveness of the recombinant genes of the present invention is demonstrated in vivo in primates.

Example 5: Heterologous prime-boost immunisation regimens with FP9.gpn and MVA.gpn elicit enhanced T cell responses.

In this example it is demonstrated that FP9.gpn and MVA.gpn elicit enhanced antigen-specific CD4+ and CD8+ T cell responses when administered to subjects in heterologous or homologous prime-boost immunisation regimens. In this example, the effect is demonstrated in mice.

Female BALB/c mice (6 – 8 weeks old) are immunised with either 1 X 10^5 PFU FP9.gpn or the 1 X 10^6 of MVA.gpn by iv. injection. Animals were boosted two weeks later with either virus in an identical manner to the initial immunisation.

Fourteen days after the booster immunisation, all mice were sacrificed by cervical dislocation and the T cell responses elicited against three CD8+ epitopes (Table A: AMQ, TTS, RGP) and a CD4+ epitope (Table A: NPP) from GPN were determined by IFN-γ ELISpot assay as described in the above examples.

Results were calculated as the sum of the number of epitope-specific IFNγ spot forming cells/million splenocytes (sfc/million). Differences between groups were determined by one-way ANOVA and a post hoc Tukey-Karamer multiple comparison test on $\log_{10}$ transformed data using GraphPad Instat version 3.05.

Results

The demonstration that immunisation with FP9.gpn and MVA.gpn in prime-boost regimes elicits enhanced T cell responses against GPN in mice is illustrated in figure 8. Female BALB/c mice were immunised iv. with FP9.gpn (FP9) or MVA.gpn (MVA)sham immunised with PBS (-) and boosted 14 days later with either virus as described above. The CD8+ T cell response was determined in splenocytes 14 days after the booster immunisation using the IFN-γ ELISpot assay. Columns represent the sum of the mean IFN-γ spot forming cells/million splenocytes ± standard deviation for 4 mice per group elicited by AMQ, TTS, RGP and NPP (see Table A).

Heterologous prime-boost immunisation with FP9.gpn and MVA.gpn in either order elicited a significantly ($P<0.001$) enhanced overall T cell response against epitopes from GPN when compared to subjects given homologous immunisations with either virus (Figure 8).

Heterologous immunisation with FP9.gpn/MVA.gpn or MVA.gpn/FP9.gpn elicits significantly higher T cell responses against GPN than homologous immunisation with FP9.gpn/FP9.gpn or MVA.gpn/MVA.gpn.

Moreover, these viruses can advantageously be used interchangeably as priming and boosting agents with each other.

Thus the efficacy of recombinant HIV genes such as the gpn gene is demonstrated, in particular when delivered by viral vector such as fowlpox or MVA based vectors.

Example 6: Heterologous prime-boost immunisation with FP9.gpn and MVA.gpn elicits enhanced CD8+ and CD4+ T cell responses against the individual component proteins of gpn

It is demonstrated that heterologous prime-boost immunisation with FP9.gpn and MVA.gpn or FP9.gpn and MVA.gpn elicits enhanced antigen-specific CD4+ and CD8+ T cell responses against the component parts of the GPN polypeptide. In this example, the demonstration is presented in mice.

Female BALB/c and C57/BL6 mice (6 – 8 weeks old) are immunised with either 1 X 10^6 PFU FP9.gpn or 1 X 10^6 PFU MVA.gpn by iv. injection. Animals are boosted two weeks later with either virus in an identical manner to the initial immunisation.

Fourteen days after the booster immunisation, all mice are sacrificed by cervical dislocation and the T cell responses elicited against a library of peptides (20 amino acids overlapping by 10 amino acids) derived from the GPN sequence determined using the IFN-γ ELISpot assay as described in the above example.

Results were calculated as the sum of the number of epitope-specific IFNγ spot forming cells/million splenocytes (sfc/million) for each peptide.

Results

Heterologous prime-boost immunisation with FP9.gpn and MVA.gpn (administered in either order) elicits enhanced T cell responses across the GPN sequence when compared to homologous immunisation regimes with the same vectors.

Moreover, these T cell responses include CD8+ and CD4+ responses, as well as being directed at epitopes derived from several regions of the GPN polypeptide.

Thus it is demonstrated that heterologous prime-boost is a highly effective application of the recombinant gene(s) of the present invention.

Example 7: Epitope Mapping of GPN in Mice

The gene encoding a gag, pol and nef polyprotein (gpn; figure 1; SEQ ID NO:11) of the Human Immunodeficiency Virus (HIV) was inserted into pox virus vectors: modified vaccinia virus Ankara (MVA.gpn) and attenuated fowlpox virus (FP9.gpn). The immune response elicited in BALB/c mice against gpn by these vectors in prime-boost immunization regimens was evaluated. These studies showed that T cell

responses can be detected in the gag, pol and nef regions of this polypeptide using pools of overlapping peptides. One aim of this study was to identify individual peptides within responding peptide pools, thereby defining the location of the responding epitopes within the gag, pol and nef regions of the polypeptide.

In this example, immunogenic epitopes within each responding peptide pool are identified, the T cell subset responsible for eliciting the identified peptide-specific IFN-γ responses is delineated and sequences corresponding to the identified epitopes are compared to other studies.

Methods

Mice and Immunisations

Female BALB/c mice (H2d; 6 – 8 weeks) were used in all experiments and kept in individually ventilated cages in accordance with the Animals (Scientific Procedure) Act 1986 of the U.K. 1 x 106 pfu of recombinant virus was administered intravenously (i.v.; 100μ into the tail vein).

Immunological Assays

ELISPOT assays were performed as described previously. A cut-off value of 3 times standard deviation of the negative control (responses >60 SFCs per million) was applied to detect positive responses.

Depletion of CD4+and CD8+ cells

A single cell suspension was prepared in PBS containing 1%FCS and incubated for 15 minutes at 4°C with either anti-CD4 or anti-CD8 MACS beads (Miltenyi Biotech®, Germany), according to the manufacturer's instructions. On completion of the incubation, cells were washed once (PBS, 1%FCS) and loaded onto a MACS column which was placed on a MACS magnet. CD4 and CD8 cell content before and after cell

subset depletion was determined by two-colour flow cytometry. CD4+-depleted splenocytes contained <1% CD4+ cells whereas >6% of CD8+ cells were still present in the splenocyte preparation following CD8+ depletion.

Peptides

A total of 119 overlapping ~20-mer peptides spanning the entire gpn-sequence were synthesized by Natural and Medical Sciences Institute (NMI; Reutlingen, Germany) (Table 1). Peptides were arranged into 6 peptide pools.

Table 1: Peptides used in this study

Peptide #	Amino Acid Sequence	Peptide Length	gag pool 1 = 20 peptides
1	MAPIVQNLQGQMVHQAISPR	20	
2	GQMVHQAISPRTLNAWVKVV	20	
3	RTLNAWVKVVEEKAFSPEVI	20	
4	EEKAFSPEVIPMFSALSEGA	20	
5	PMFSALSEGATPQDLNTML	19	
6	ATPQDLNTMLNTVGGHQAAM	20	
7	NTVGGHQAAMQMLKETI	17	
8	AAMQMLKETINEEAAEWDRL	20	
9	NEEAAEWDRLHPVHAGPIA	19	
10	LHPVHAGPIAPGQMREPR	18	
11	IAPGQMREPRGSDIAGTTSTL	21	
12	SDIAGTTSTLQEQIGWM	17	
13	STLQEQIGWMTNNPPIPV	18	
14	WMTNNPPIPVGEIYKRWIIL	20	
15	GEIYKRWIILGLNKIVRMY	19	
16	LGLNKIVRMYSPTSILDIRQ	20	
17	SPTSILDIRQGPKEPFRDYV	20	

18	GPKEPFRDYVDRFYKTLRA	19	
19	VDRFYKTLRAEQASQEVKNW	20	
20	EQASQEVKNWMTETLLVQNA	20	
21	MTETLLVQNANPDCKTILKA	20	**gag pool 2 = 20**
22	NPDCKTILKALGPAATLEEM	20	**peptides**
23	LGPAATLEEMMTACQGV	17	
24	EEMMTACQGVGGPGHKARVL	20	
25	GGPGHKARVLMAARASVL	18	
26	VLMAARASVLSGGELDRWEK	20	
27	SGGELDRWEKIRLRPGGKKK	20	
28	IRLRPGGKKKYKLKHIVWA	19	
29	KYKLKHIVWASRELERFAV	19	
30	ASRELERFAVNPGLLETSEGCR	22	
31	GLLETSEGCRQILGQLQPSL	20	
32	RQILGQLQPSLQTGSEELR	19	
33	SLQTGSEELRSLYNTVATLY	20	
34	SLYNTVATLYCVHQRIEVK	19	
35	YCVHQRIEVKDTKEALEKI	19	
36	KDTKEALEKIEEEQNKSKKK	20	
37	EEEQNKSKKKAQQAAADTGN	20	
38	AQQAAADTGNSSQVSQNY	18	
39	GNSSQVSQNYTPDKKHQK	18	
40	NYTPDKKHQKEPPFLWMGY	19	

41	KEPPFLWMGYELHPDKWTVQ	20	**pol pool 1 = 23**
42	ELHPDKWTVQPIVLPEKDSW	20	**peptides**
43	PIVLPEKDSWTVNDIQKLV	19	
44	WTVNDIQKLVGKLNWASQIY	20	
45	GKLNWASQIYAGIKVKQLCK	20	

46	AGIKVKQLCKLLRGTKAL	18	
47	CKLLRGTKALTEVIPLTEEA	20	
48	TEVIPLTEEAELELAENREI	20	
49	ELELAENREILKEPVHGVYY	20	
50	LKEPVHGVYYDPSKDLIAEI	20	
51	DPSKDLIAEIQKQGQGQWTY	20	
52	IQKQGQGQWTYQIYQEPFK	19	
53	TYQIYQEPFKNLKTGKYARM	20	
54	NLKTGKYARMRGAHTNDVKQ	20	
55	RGAHTNDVKQLTEAVQKIA	19	
56	KQLTEAVQKIATESIVIWGK	20	
57	ATESIVIWGKTPKFKLPIQK	20	
58	TPKFKLPIQKETWEAWWTEY	20	
59	ETWEAWWTEYWQATWIPEW	19	
60	YWQATWIPEWEFVNTPPLVK	20	
61	EFVNTPPLVKLWYQLEKEPI	20	
62	LWYQLEKEPIVGAETFPI	18	
63	PIVGAETFPISPIETVPVKL	20	
64	SPIETVPVKLKPGMDGPKVK	20	**pol pool 2 = 23 peptides**
65	KPGMDGPKVKQWPLTEEKIK	20	
66	KQWPLTEEKIKALVEICTEM	20	
67	KALVEICTEMEKEGKISKI	19	
68	MEKEGKISKIGPENPYNTPV	20	
69	GPENPYNTPVFAIKKKDSTK	20	
70	FAIKKKDSTKWRKLVDFREL	20	
71	WRKLVDFRELNKRTQDFWEV	20	
72	NKRTQDFWEVQLGIPHPAGL	20	
73	VQLGIPHPAGLKKKKSVTVL	20	
74	LKKKKSVTVLDVGDAYFSV	19	
75	LDVGDAYFSVPLDKDFRKY	19	

76	VPLDKDFRKYTAFTIPSI	18	
77	KYTAFTIPSINNETPGIRYQ	20	
78	NNETPGIRYQYNVLPQGWK	19	
79	YQYNVLPQGWKGSPAIFQ	18	
80	GWKGSPAIFQSSMTKILEPF	20	
81	SSMTKILEPFRKQNPDIVIY	20	
82	RKQNPDIVIYQYMDDLYV	18	
83	IYQYMDDLYVGSDLEIGQHR	20	
84	GSDLEIGQHRTKIEELRQHL	20	
85	TKIEELRQHLLRWGFTTPDK	20	
86	LRWGFTTPDKKHQKEPPFLV	20	

87	KHQKEPPFLVWKFDSRLAFH	20	**nef pool 1 = 16**
88	WKFDSRLAFHHMARELHPEY	20	**peptides**
89	HMARELHPEYYKDCDPEKEV	20	
90	YKDCDPEKEVLVWKFDA	17	
91	KEVLVWKFDANEGENNSLLH	20	
92	NEGENNSLLHPMSLHGM	17	
93	LLHPMSLHGMDDPEKEVPEK	20	
94	DDPEKEVPEKVEEANEGENG	20	
95	VEEANEGENGPGIRYPLTF	19	
96	GPGIRYPLTFGWCFKLVPV	19	
97	FGWCFKLVPVEPEKVEEWQ	19	
98	VEPEKVEEWQNYTPGPGIRY	20	
99	NYTPGPGIRYQKRQDILDLW	20	
100	YQKRQDILDLWVYHTQGYF	19	
101	LWVYHTQGYFPDWQNYTPEGL	21	
102	DWQNYTPEGLIYSQKRQDI	19	
103	LIYSQKRQDIPMTYKAALDL	20	**nef pool 2 = 17**
104	PMTYKAALDLSHFLKEKGGL	20	**peptides**

105	SHFLKEKGGLEGLIYSPQV	19	
106	LEGLIYSPQVPLRPMTYKAA	20	
107	PLRPMTYKAADCAWLEAQ	18	
108	AADCAWLEAQEEEEVGFPVR	20	
109	EEEEVGFPVRPQVPLRNTAA	20	
110	PQVPLRNTAANNADCAWLA	19	
111	ANNADCAWLADGVGAVSRDL	20	
112	DGVGAVSRDLEKHGAITSSNTA	22	
113	HGAITSSNTAANNRRAEPAA	20	
114	ANNRRAEPAADGVGAMGGKW	20	
115	DGVGAMGGKWSKRSVVGW	18	
116	KWSKRSVVGWPTVRERMRRA	20	
117	PTVRERMRRAEPARGPGRAF	20	
118	EPARGPGRAFVTIYPYDV	18	
119	AFVTIYPYDVPDYA	14	
AMQ	AMQMLKETI	9	Immunodominant control
RGP	RGPGRAFVTI	10	Reporter epitope control

Results

Identification of immunogenic epitopes within each responding peptide pool

Individual epitope mapping of responding peptide pools was conducted by IFN-γ.

ELISPOT assay was performed using overlapping peptides from each pool (see Figure 13). Figure 13 shows IFN-γ responses elicited against peptide pools and overlapping

peptide fragments covering the entire gpn-sequence (with markers). Groups of female BALB/c mice ($H2^d$; n=4 mice) were immunised intravenously (i.v.) with 1 x 10^6 plaque forming units (pfu) of MVA.gpn. Fourteen days later, mice were boosted by i.v. administration of 1 x 10^6 pfu of FP9.gpn. Fourteen days after boosting, spleens were removed, pooled (n=4 spleens) and the number of IFN-γ spot forming cells (SFC) per million splenocytes was determined by IFN-γ ELISPOT. Columns represent the number of SFC/million.

In summary these results indicate that the following individual peptides in responding peptide pools were identified:

In peptide pool gag1: peptide 7: NTVGGHQAAMQMLKETI
peptide 8: AAMQMLKETINEEAAEWDRL
peptide 15: GEIYKRWIILGLNKIVRMY

In peptide pool pol1: peptide 50: LKEPVHGVYYDPSKDLIAEI

In peptide pool pol2: peptide 66: KQWPLTEEKIKALVEICTEM
peptide 85: TKIEELRQHLLRWGFTTPDK

In peptide pool nef2: peptide118: EPARGPGRAFVTIYPYDV

The underlined sequence in nef2 corresponds to the reporter epitope that was added to provide a measure of the induction of IFN-γ responses. This response in the penultimate peptide, 118, indicates that the entire GPN polyprotein is being expressed. However the IFN-γ response detected in peptide pool nef2 was not due to epitope sequences derived from native nef but due to the inserted reporter epitope. Example 8 contains very few CD8+ T cell epitopes in nef for BALB/c mice (H-2d) so the lack of response in nef is not surprising.

Determination of the T cell subset responsible for eliciting the identified peptide-specific IFN-γ responses

Figure 14 shows IFN-γ responses elicited against selected peptides from gpn-sequence. Groups of female BALB/c mice ($H2^d$; n=4 mice) were immunised intravenously (i.v.) with 1 x 10^6 plaque forming units (pfu) of MVA.gpn. Fourteen days later, mice were boosted by i.v. administration of 1 x 10^6 pfu of FP9.gpn. Fourteen days after boosting, spleens were removed, pooled (n=4 spleens) and either left untreated, depleted of $CD4^+$ cells or depleted of $CD8^+$ cells as indicated. The number of IFN-γ spot forming cells (SFC) per million splenocytes was determined by IFN-γ ELISPOT. Columns represent the number of SFC/million.

CD4+ cell depletion studies demonstrated a dramatic reduction in IFN-γ secretion following *ex vivo* stimulation with peptides 15 and 66, whereas a moderate reduction was observed after stimulation with peptide 118. These findings suggest that $CD4^+$ T cells elicit IFN-γ responses against peptides 15, 66 and 118 (see figure 14).

A partial reduction in the IFN-γ response against peptides 7, 50, 85 and 118 was observed following depletion of $CD8^+$ T cells. The partial reduction in IFN-γ responses observed against these peptides indicates that they are mediated by $CD8^+$ T cells (see figure 14).

Comparison of sequences corresponding to the identified epitopes with other described epitopes

To determine whether the peptide sequences identified in this study have been defined previously , they were compared with those in the Los Alamos HIV sequence data base (mouse; see example 8 (BALB/c; $H2^d$))

Epitopes within the sequences corresponding to peptides 7,8,15 and 118 have been previously identified in BALB/c mice according to Example 8, whereas epitopes within sequences corresponding to peptides 50, 66 and peptide 85 have not been previously described.

Epitopes within the sequences corresponding to all seven peptides described in this report have been identified as immunogenic in HIV infected humans as shown in Table 2.

Table 2: Results from Los Alamos HIV data base search.

Peptide #	Mouse (BALB/c; $H2^d$)	Human
7	MHC class I K^d-restricted	MHC class I-restricted
8	MHC class I K^d-restricted	MHC class I-restricted
15	MHC class II-restricted	MHC class II-restricted
50	this study	MHC class I-restricted
66	this study	MHC class I-restricted
85	this study	MHC class I-restricted
118	MHC class I Dd-restricted MHC class II IA^d-restricted	MHC class I-restricted MHC class II-restricted

These studies indicate that heterologous prime-boost immunisation regimens using MVA.gpn and FP9.gpn elicit potent $CD4^+$ and $CD8^+$ IFN-γ secreting T cell responses against the gag and pol regions of the gpn-molecule in BALB/c mice. In common with previous studies conducted with BALB/c mice, no immunogenic epitopes were identified in the nef molecule.

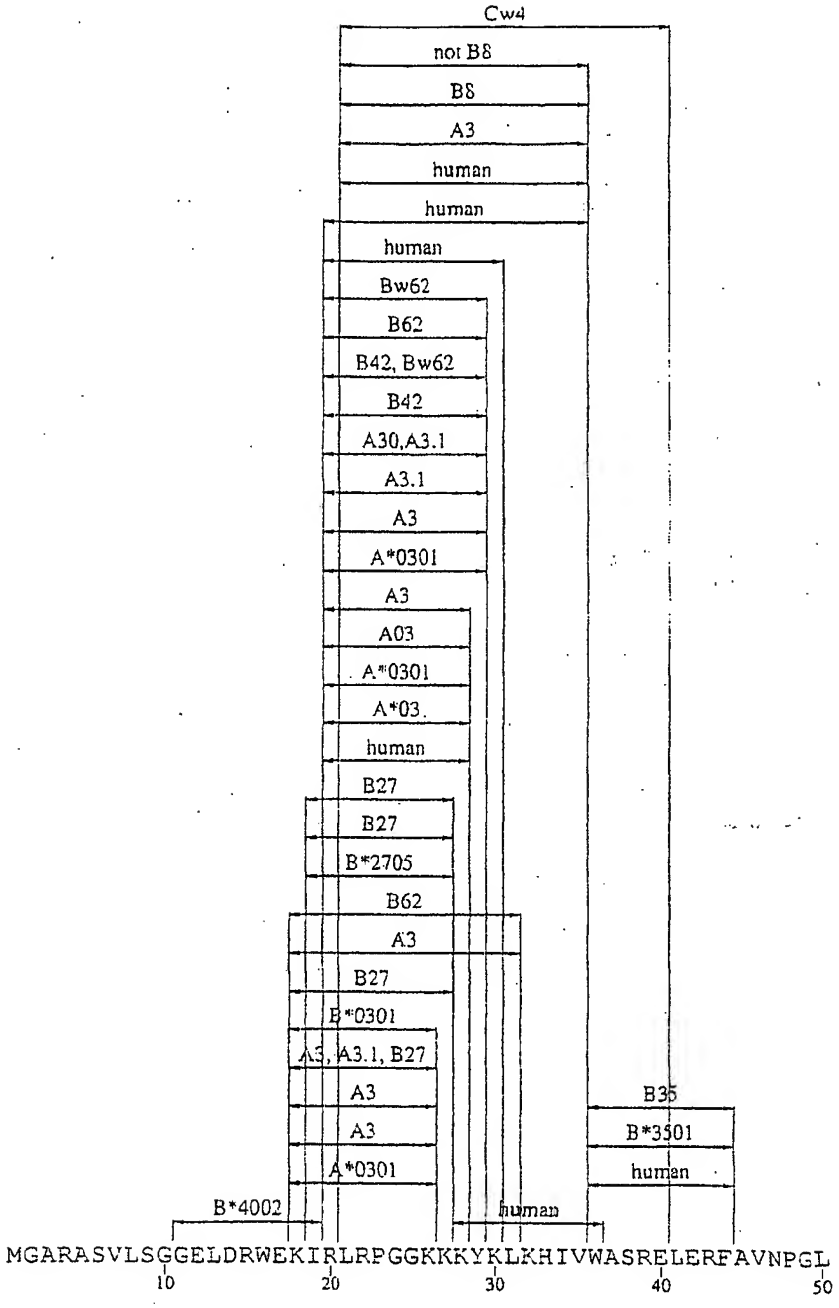
All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the present invention will be apparent to those skilled in the art without departing from the scope and spirit of the present invention. Although the present invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in biochemistry and biotechnology or related fields are intended to be within the scope of the following claims.

Example 8: Maps of CTL epitope locations plotted by protein

This example presents preferred CTL epitopes following HIV Molecular Immunology 2002: Maps of CTL Epitope Locations Plotted by Protein; Theoretical Biology & Biophysics, Los Alamos National Laboratory, August 7, 2003. Linear CTL epitopes less than 22 amino acids long are shown. Also shown are T-Helper epitope maps of the preferred T-Helper epitopes.

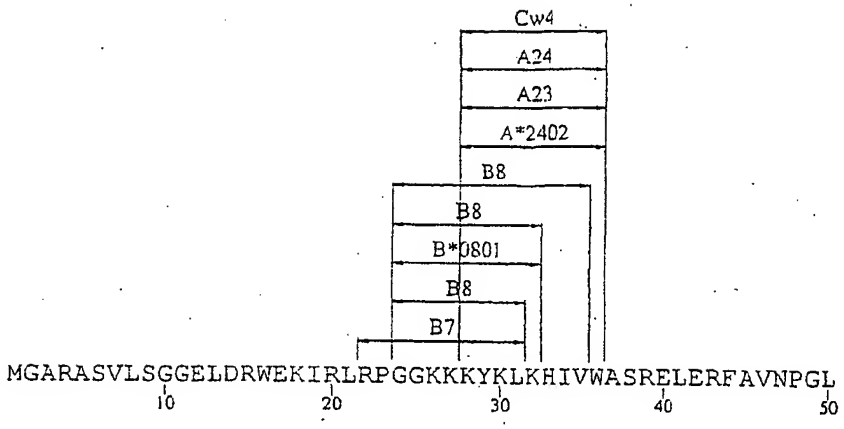
1 p17 CTL Epitope Map

p17 CTL Map aa 1-50 1/2



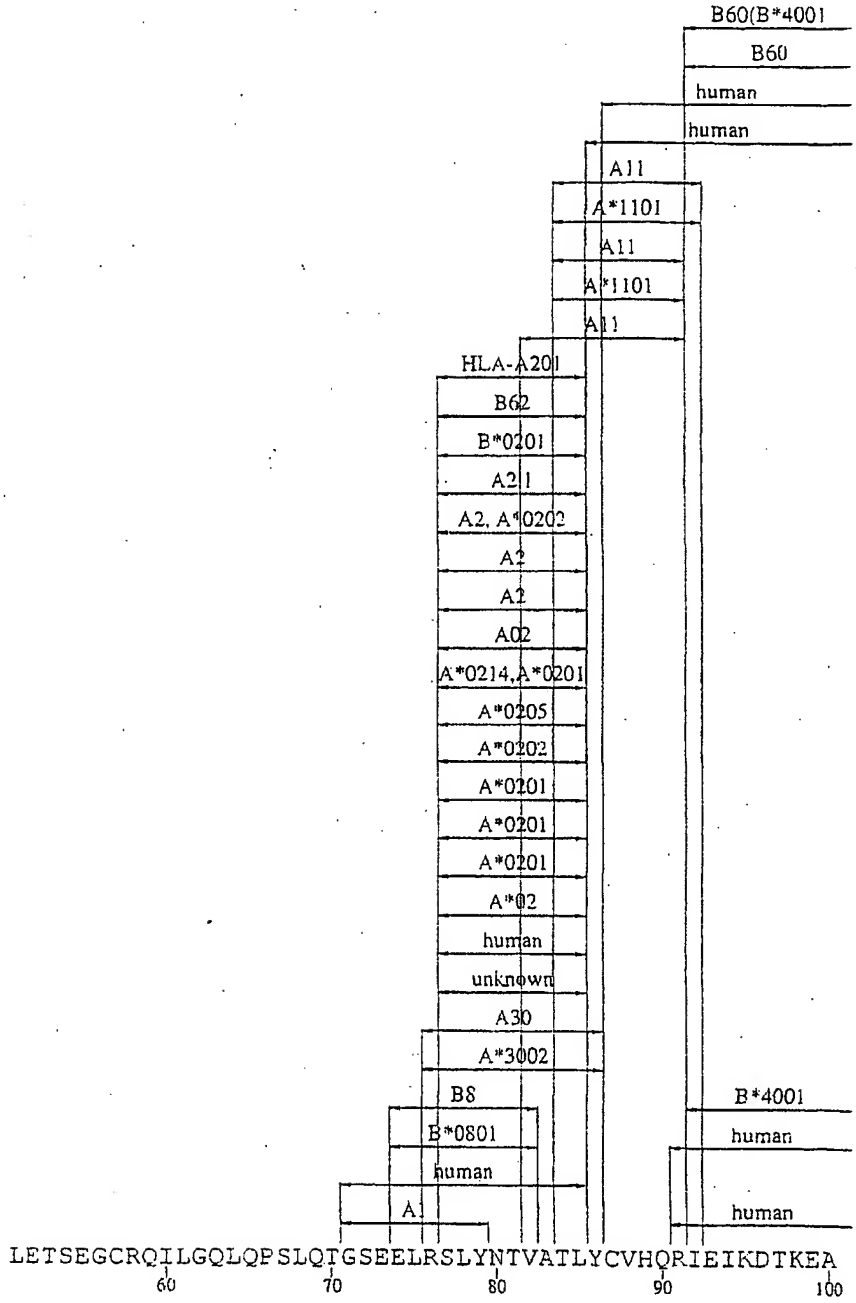
p17 CTL Epitope Map

p17 CTL Map aa 1-50 2/2



p17 CTL Epitope Map

p17 CTL Map aa 51–100 1/2



p17 CTL Epitope Map

p17 CTL Map aa 51–100 2/2

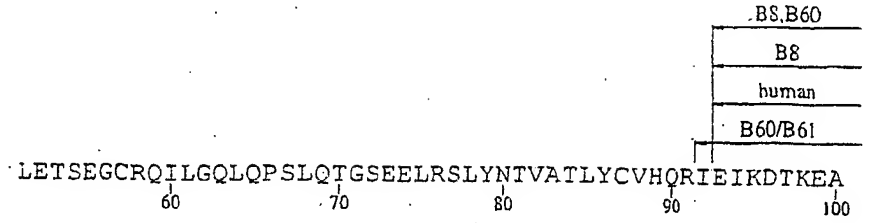
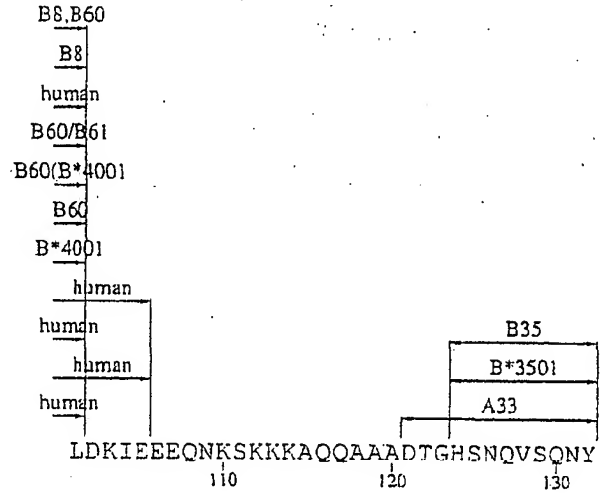
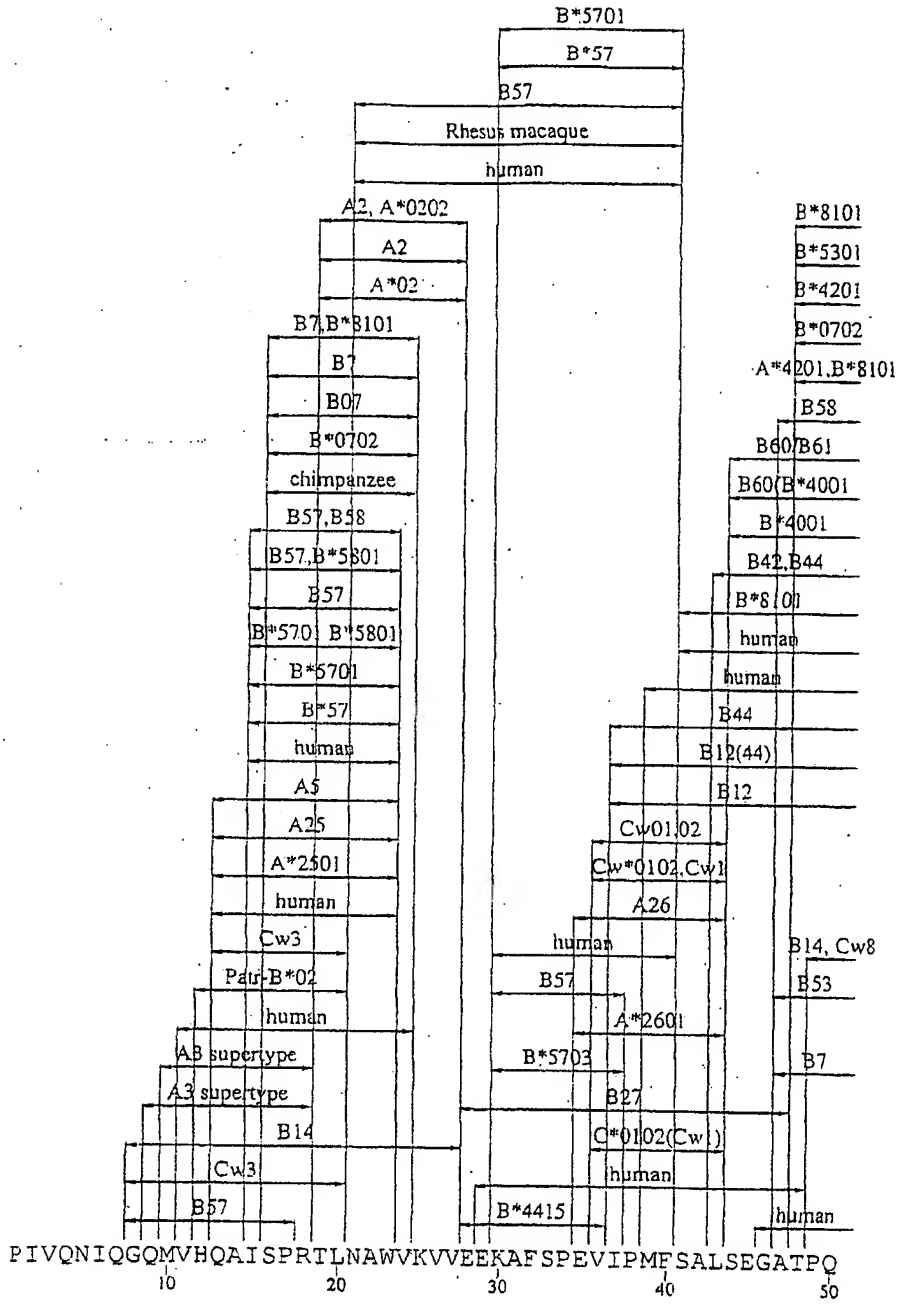


Figure 5: p17 CTL Map aa 101–132



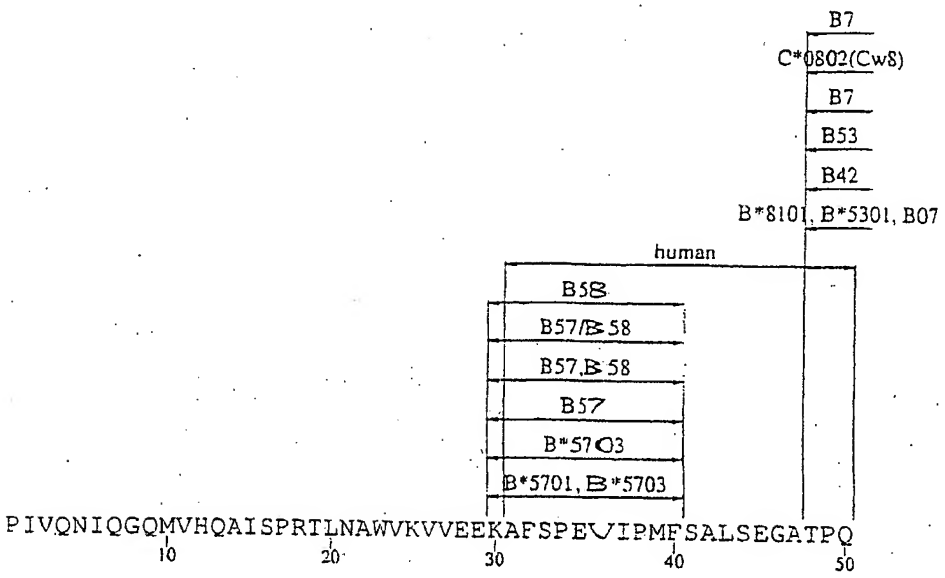
2 p24 CTL Epitope Map

p24 CTL Map aa 1–50 1/2



p24 CTL Epitope Map

p24 CTL Map aa 1–50 2/2



p24 CTL Epitope Map

p24 CTL Map aa 51–100 1/2

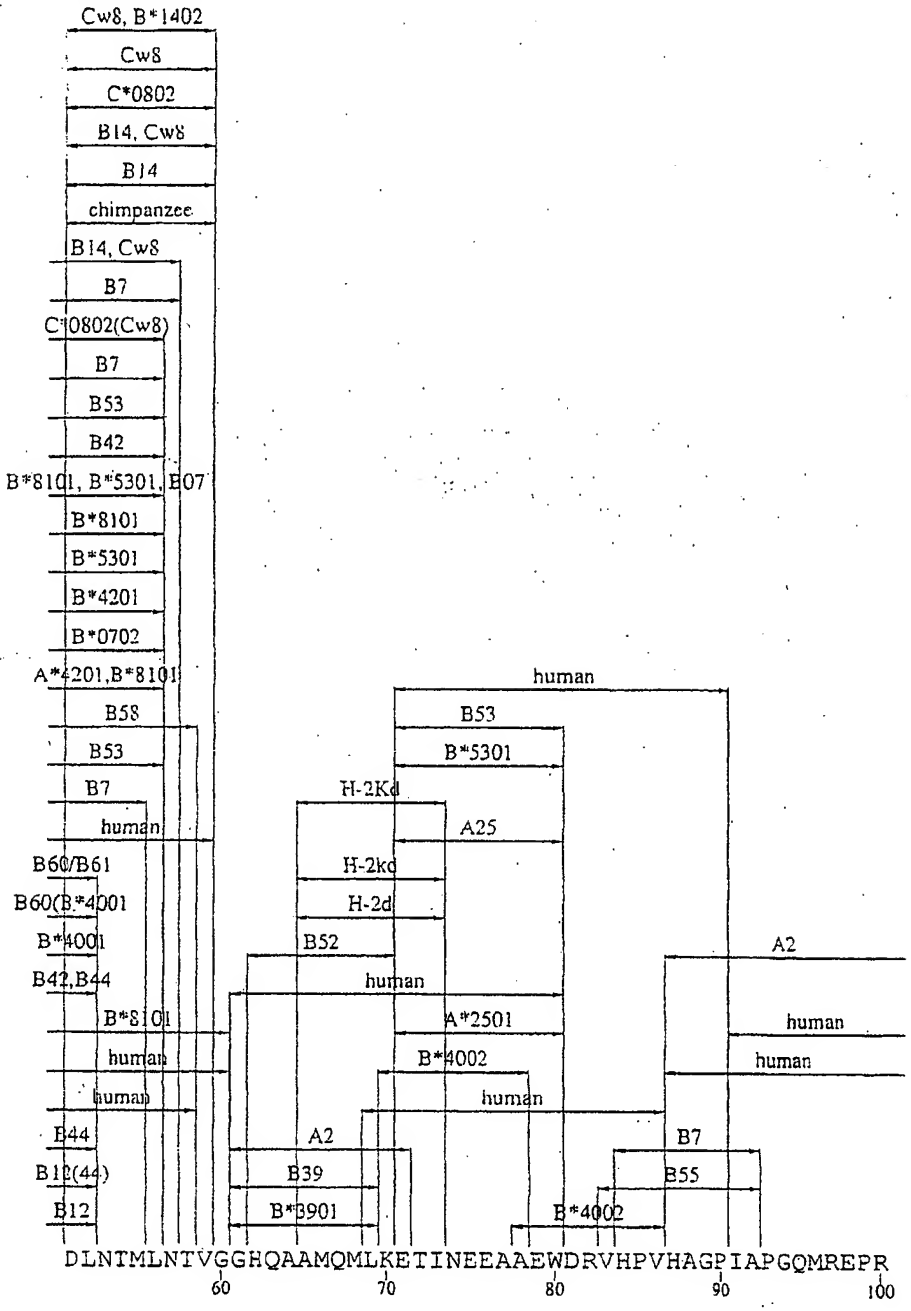
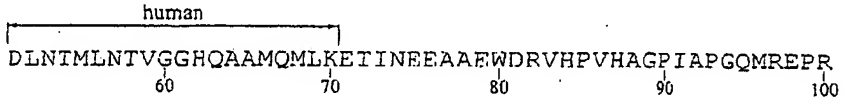
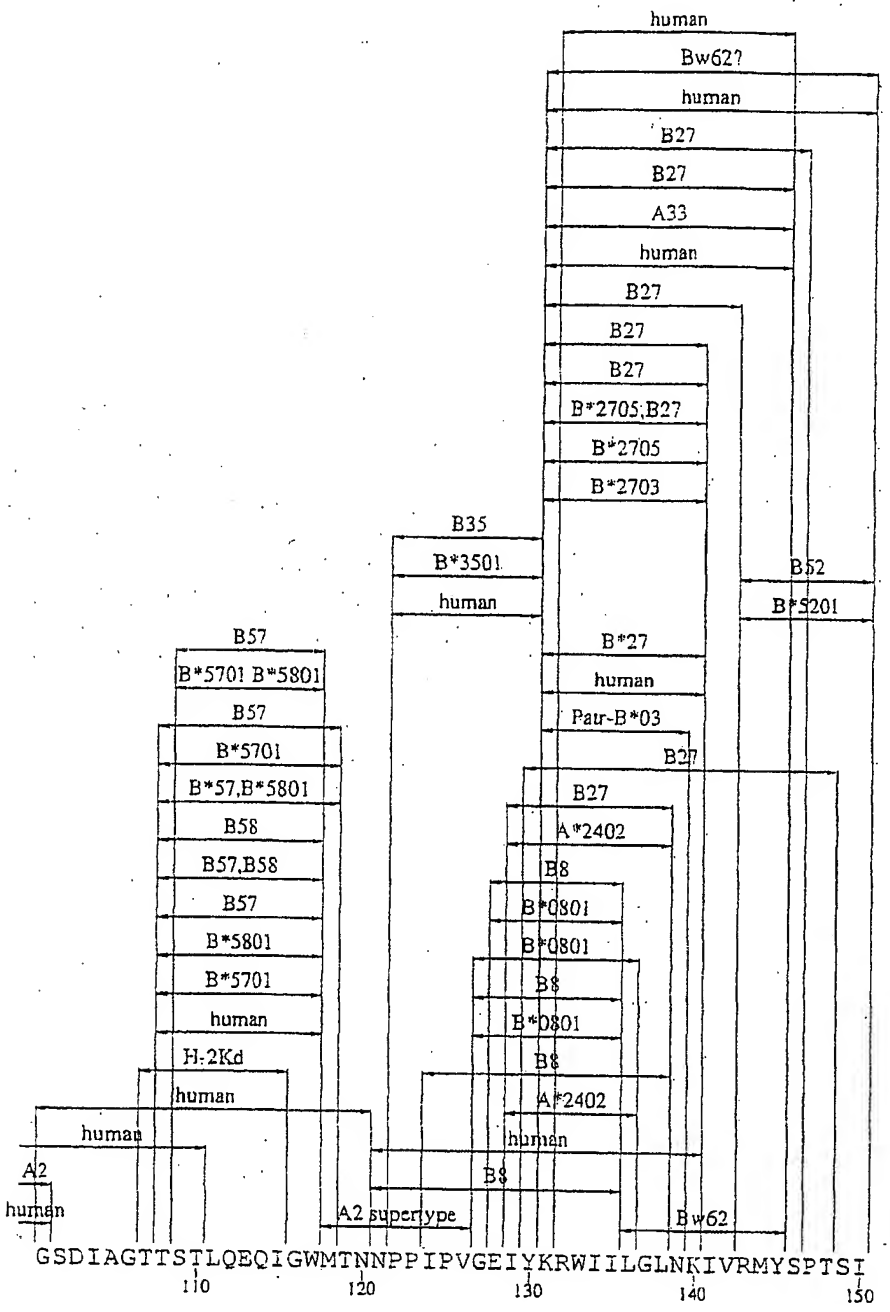


Figure 9: p24 CTL Map aa 51–100 2/2



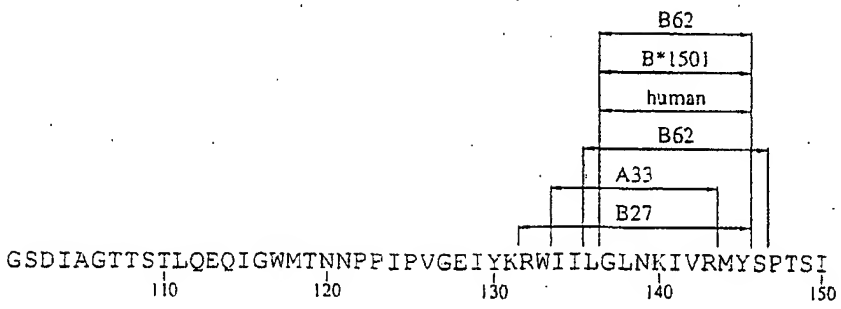
p24 CTL Epitope Map

p24 CTL Map aa 101–150 1/2



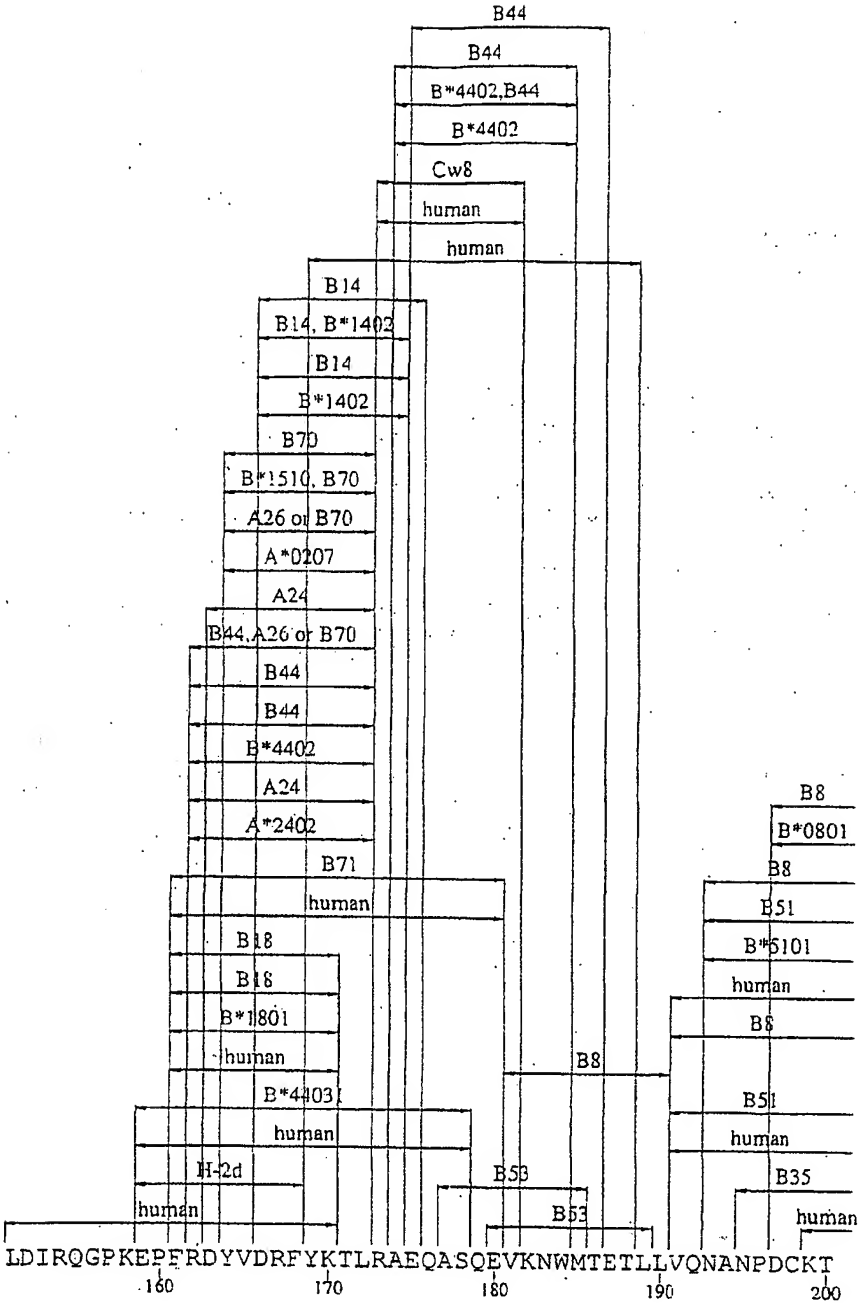
p24 CTL Epitope Map

p24 CTL Map aa 101-150 2/2



p24 CTL Epitope Map

p24 CTL Map aa 151–200 1/2



p24 CTL Epitope Map

p24 CTL Map aa 151–200 2/2

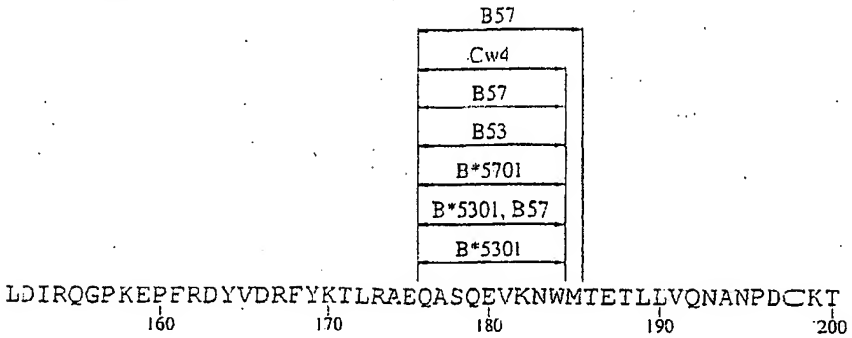
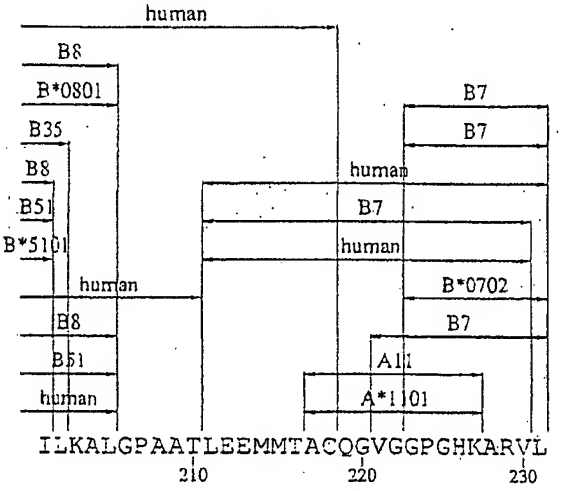


Figure 14: p24 CTL Map aa 201–231



5 RT CTL Epitope Map

RT CTL Map aa 1–50

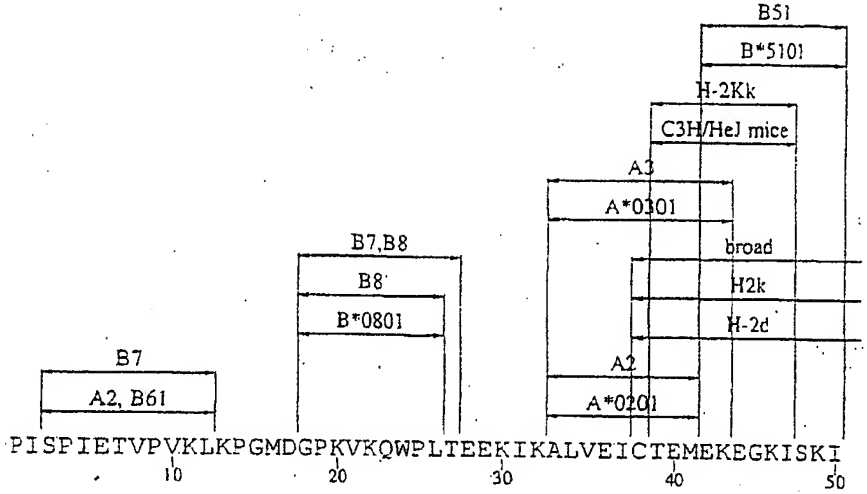
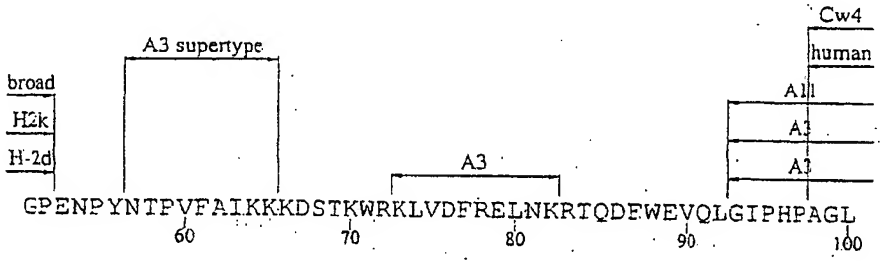
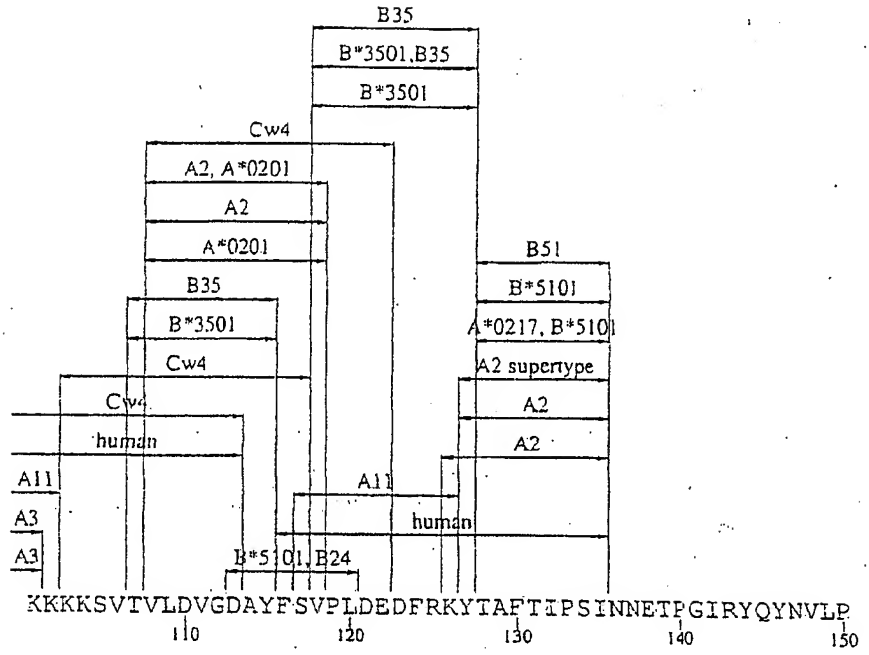


Figure 21: RT CTL Map aa 51–100



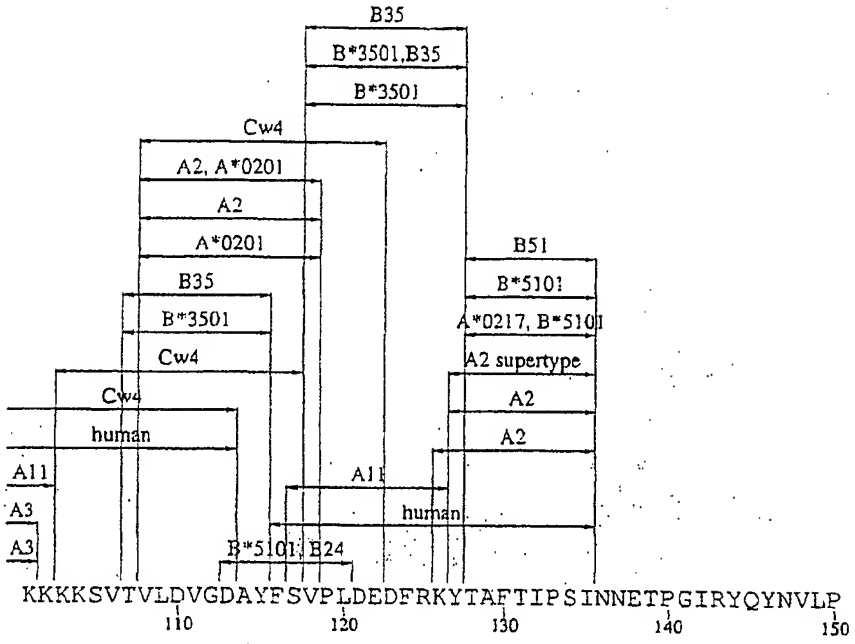
RT CTL Epitope Map

RT CTL Map aa 101–150



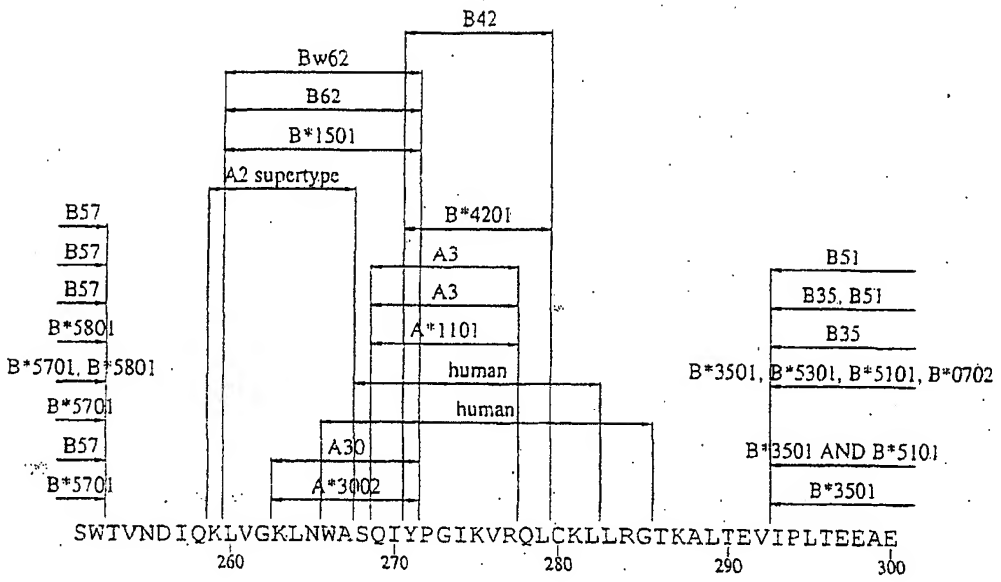
RT CTL Epitope Map

RT CTL Map aa 101–150



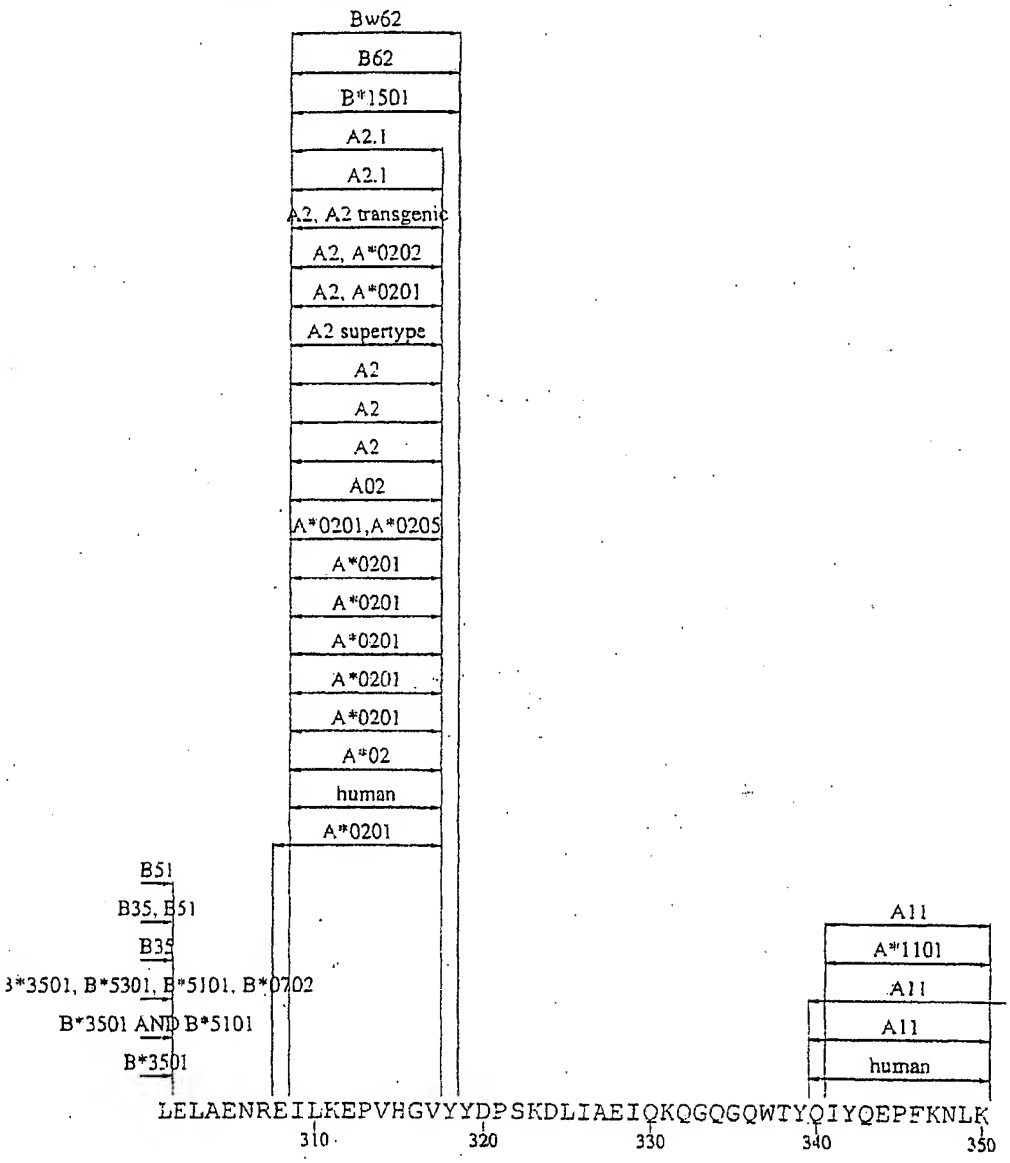
RT CTL Epitope Map

RT CTL Map aa 251–300



RT CTL Epitope Map

RT CTL Map aa 301–350



RT CTL Map aa 351–400

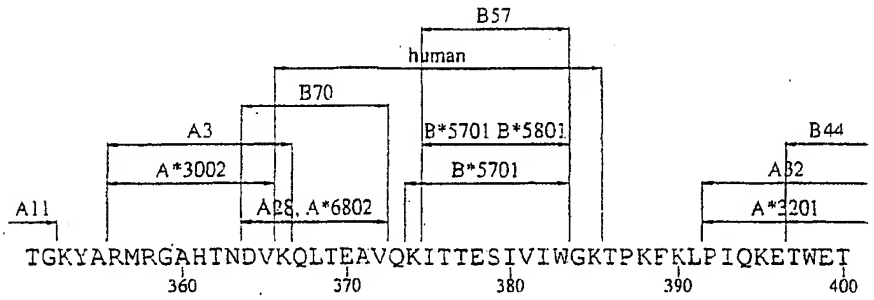


Figure 28: RT CTL Map aa 401–450

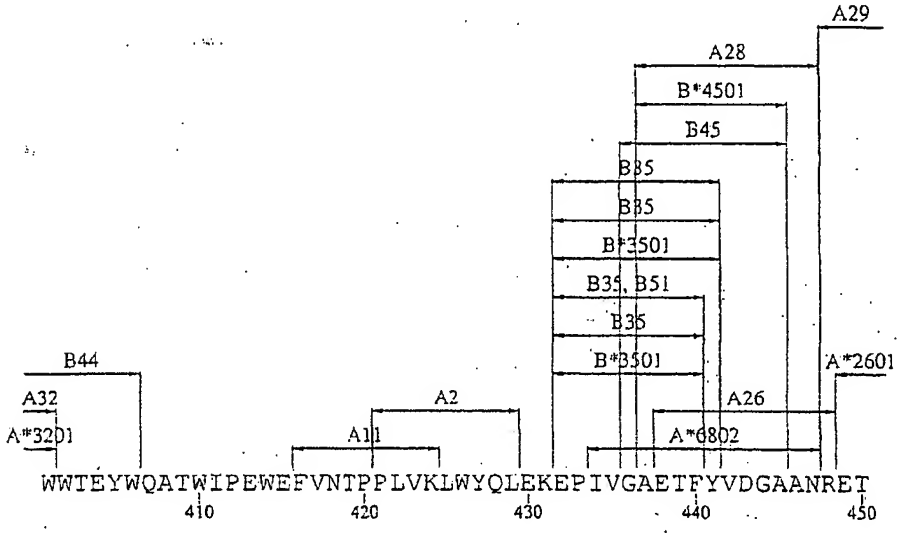
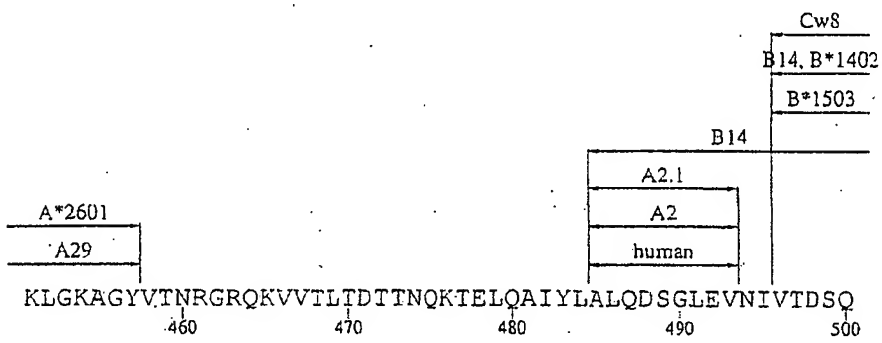


Figure 29: RT CTL Map aa 451–500



RT CTL Epitope Map

RT CTL Map aa 501–550

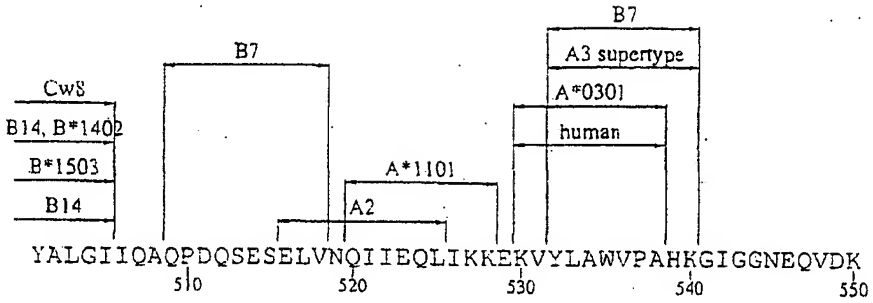
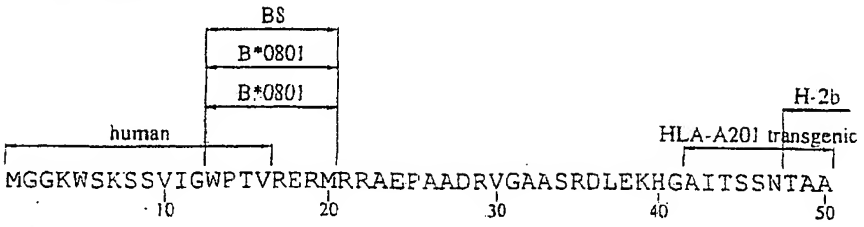


Figure 31: RT CTL Map aa 551–560

LVSAGIRKVL
560

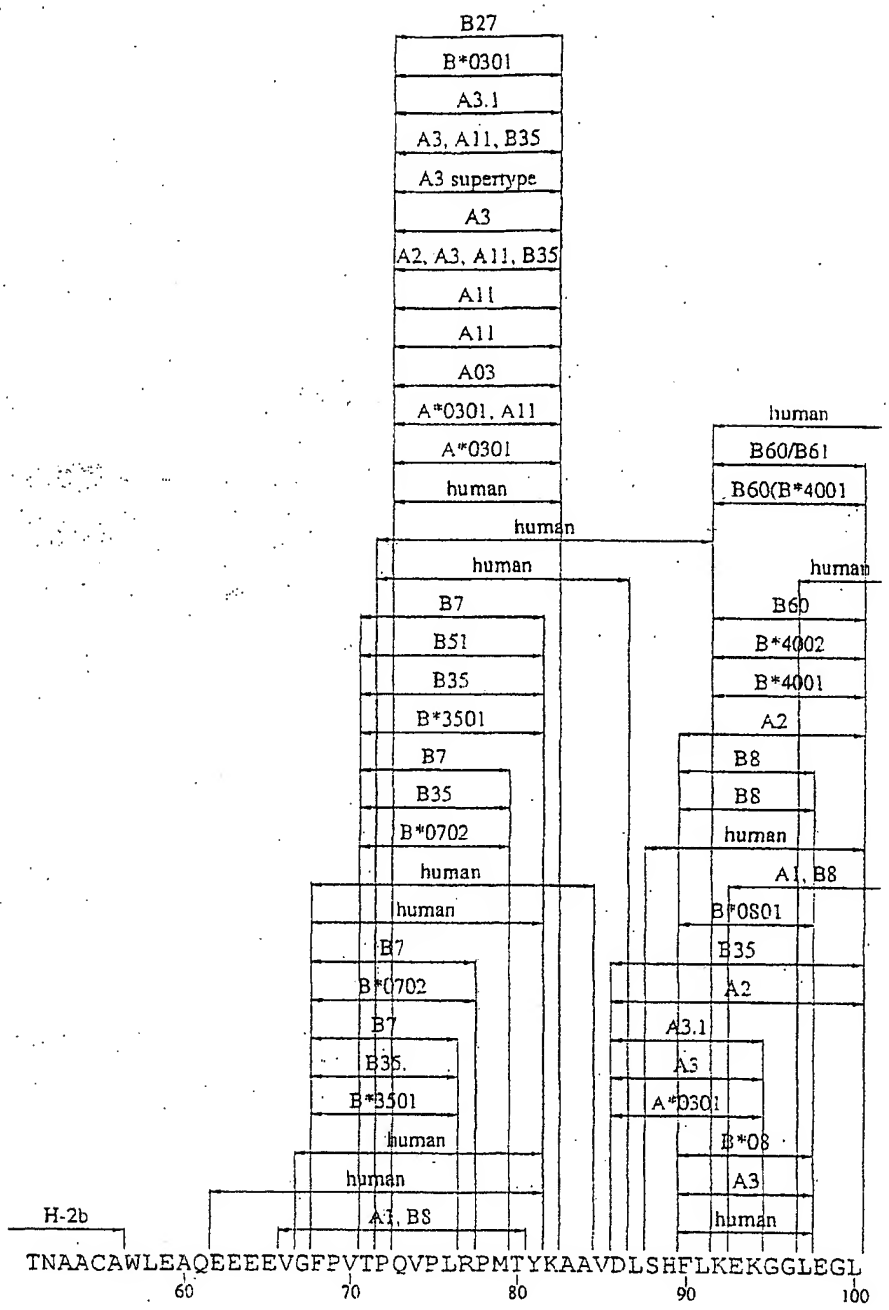
13 Nef CTL Epitope Map

Nef CTL Map aa 1–50



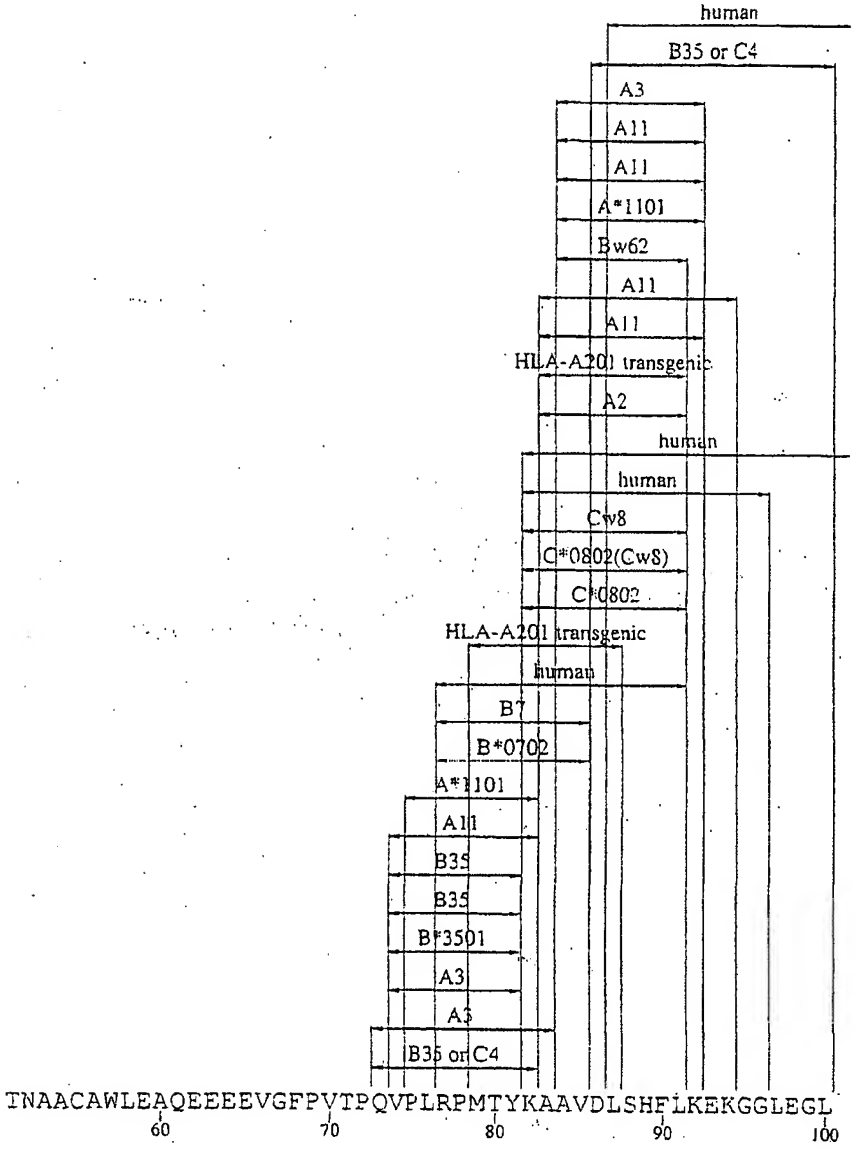
Nef CTL Epitope Map

Nef CTL Map aa 51–100 1/3



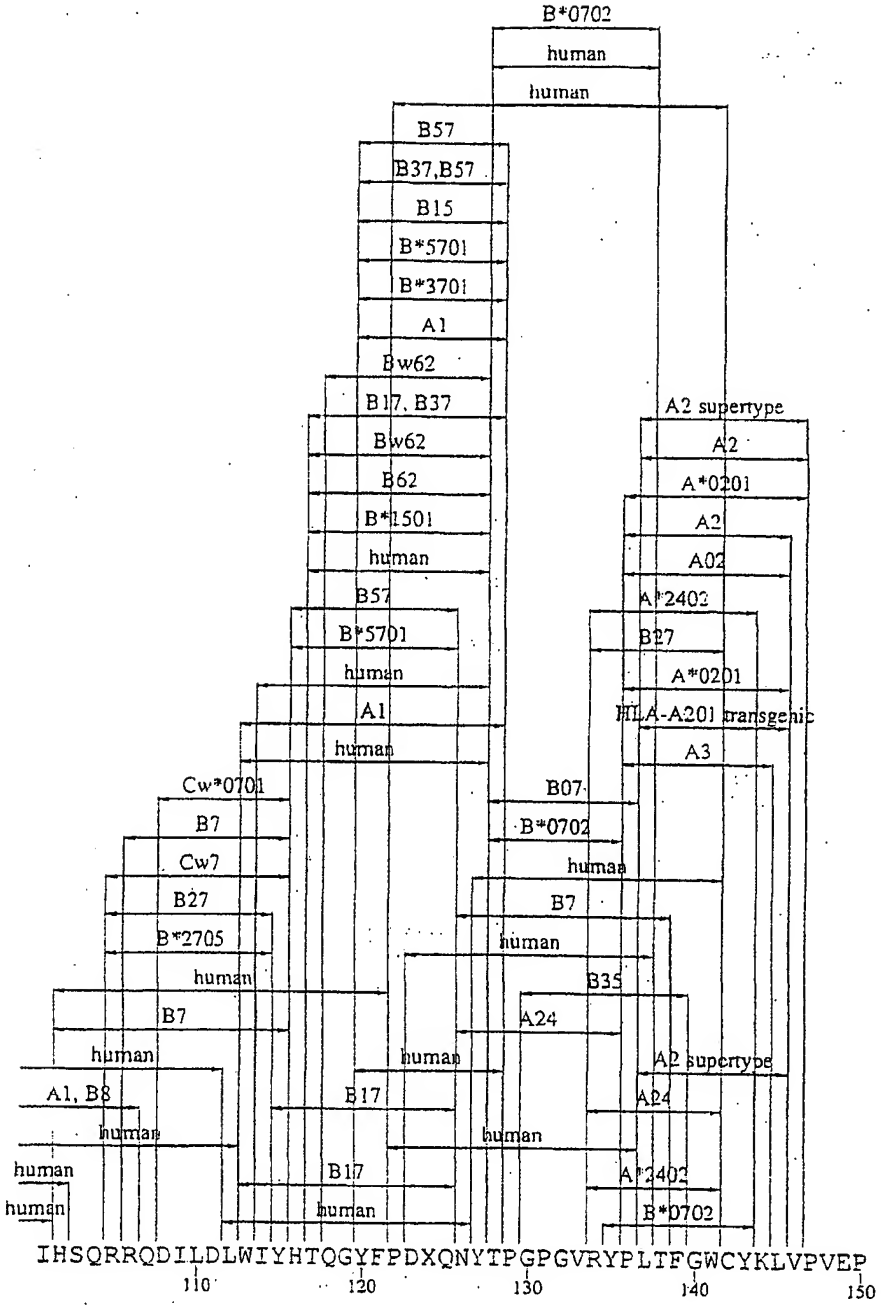
Nef CTL Epitope Map

Nef CTL Map aa 51–100 2/3



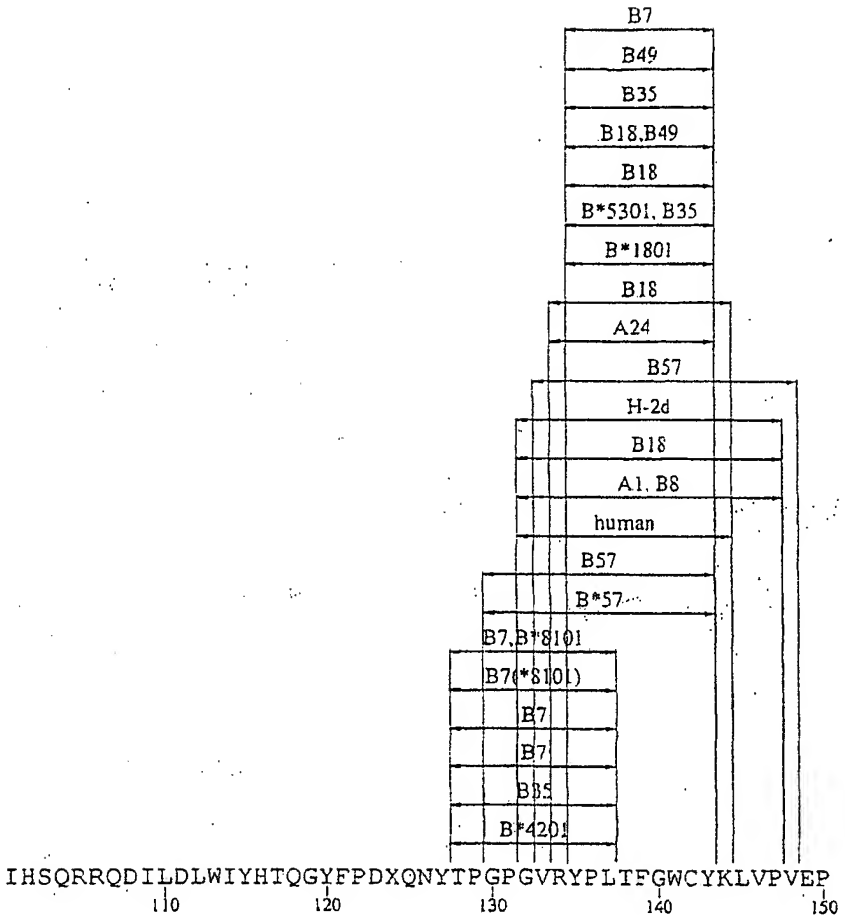
Nef CTL Epitope Map

Nef CTL Map aa 51–100 3/3



Nef CTL Epitope Map

Nef CTL Map aa 101-150 1/2



Nef CTL Epitope Map

Nef CTL Map aa 101–150 2/2

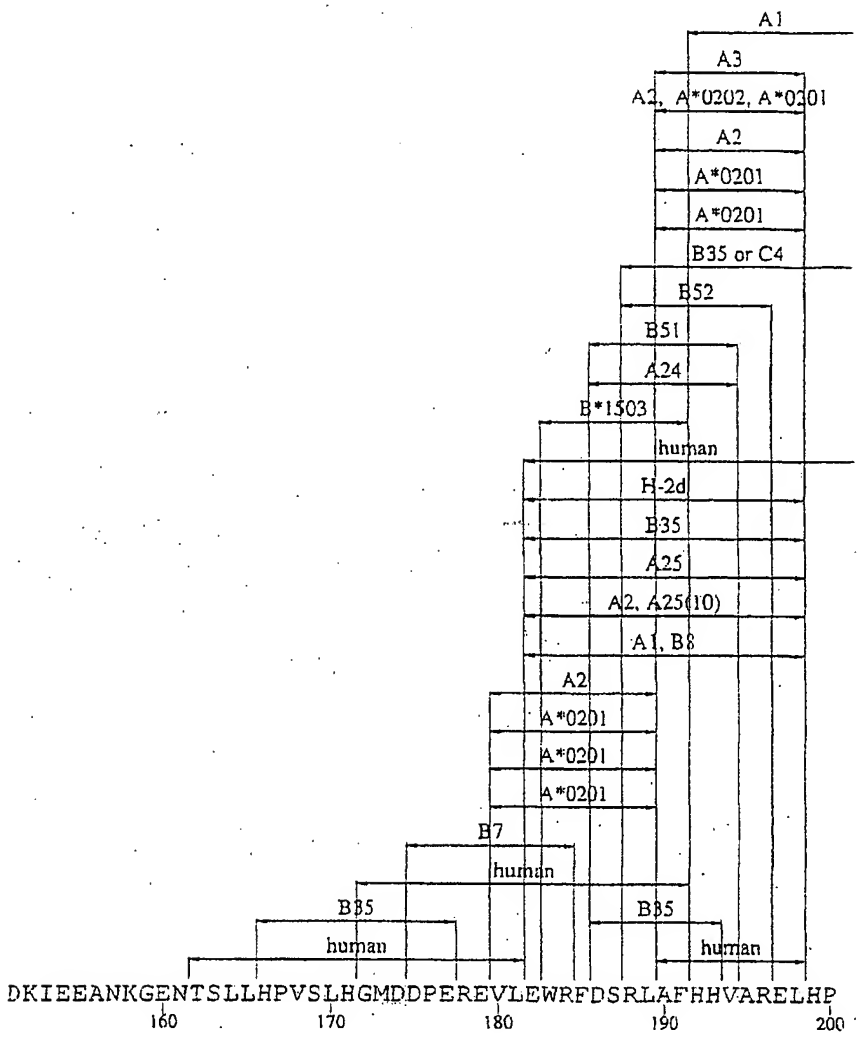
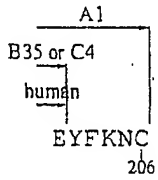
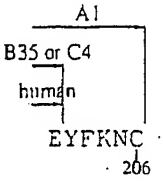


Figure 78: Nef CTL Map aa 151–200



Nef CTL Epitope Map

. Nef CTL Map aa 201-206



1 p17 T-Helper Epitope Map

p17 T-Helper Map aa 1–50

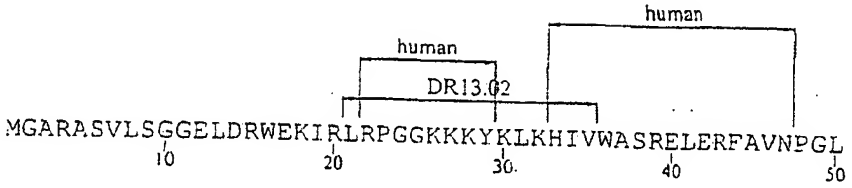


Figure 2: p17 T-Helper Map aa 51–100

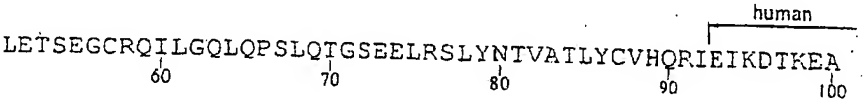
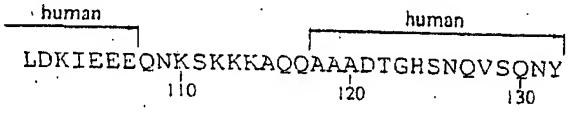


Figure 3: p17 T-Helper Map aa 101–132



2 p24 T-Helper Epitope Map

p24 T-Helper Map aa 1–50

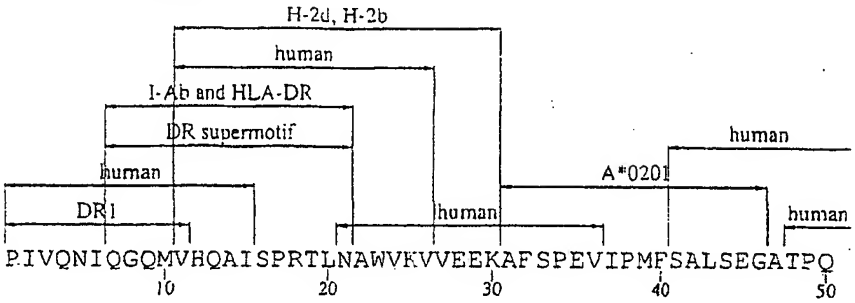


Figure 5: p24 T-Helper Map aa 51–100

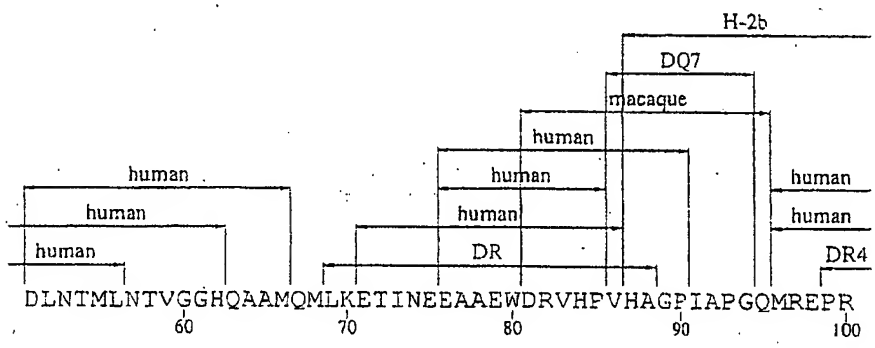
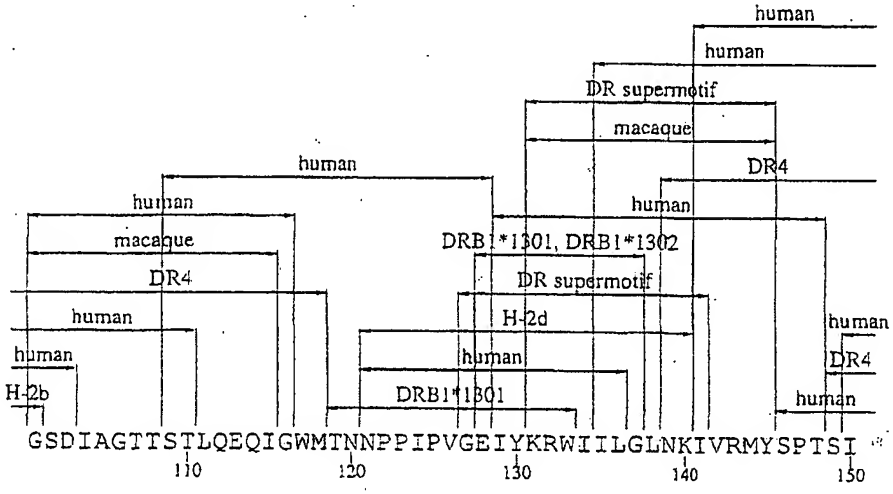


Figure 6: p24 T-Helper Map aa 101–150



p24 T-Helper Epitope Map

p24 T-Helper Map aa 151–200

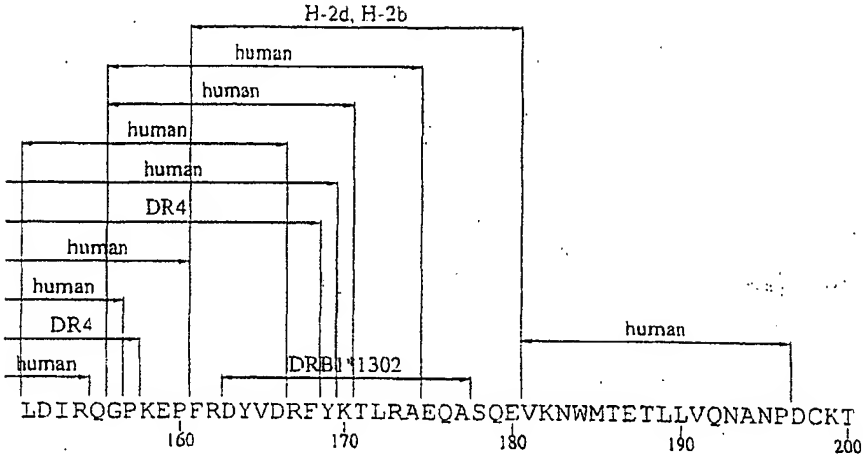
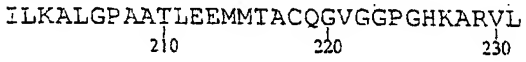


Figure 8: p24 T-Helper Map aa 201–231



5 RT T-Helper Epitope Map

RT T-Helper Map aa 1–50

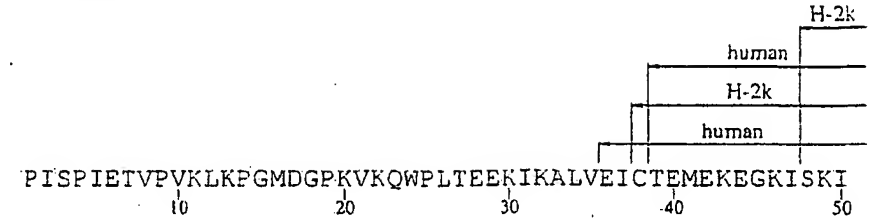


Figure 15: RT T-Helper Map aa 51–100

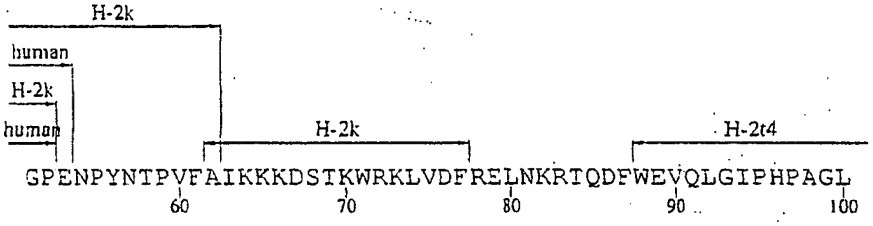


Figure 16: RT T-Helper Map aa 101–150

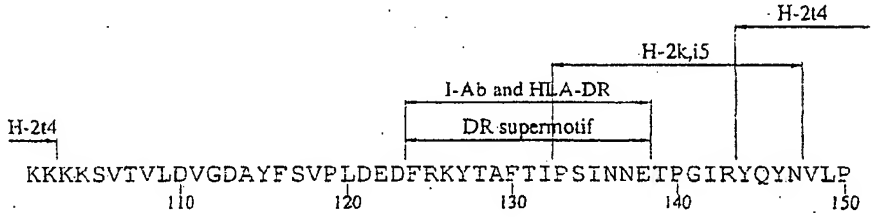


Figure 17: RT T-Helper Map aa 151–200

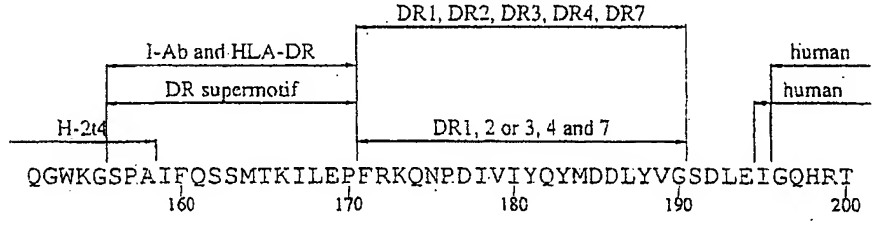
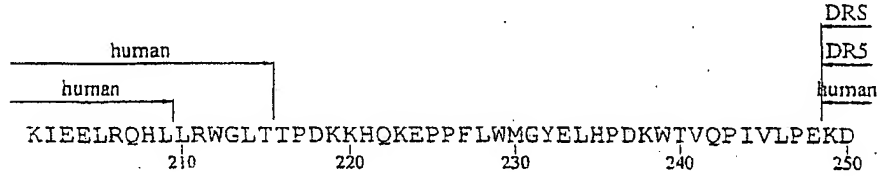


Figure 18: RT T-Helper Map aa 201–250



RT T-Helper Epitope Map

RT T-Helper Map aa 251–300

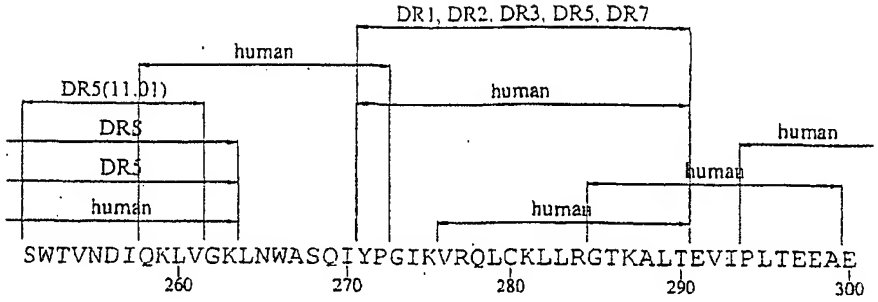


Figure 20: RT T-Helper Map aa 301–350

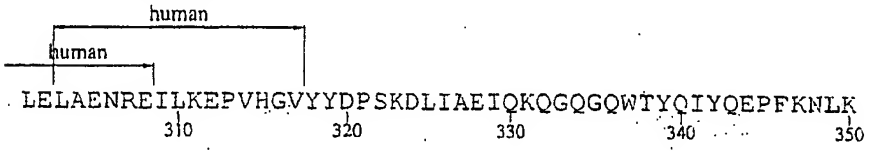


Figure 21: RT T-Helper Map aa 351–400

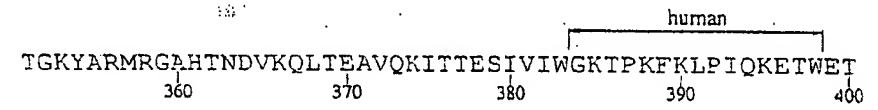


Figure 22: RT T-Helper Map aa 401–450

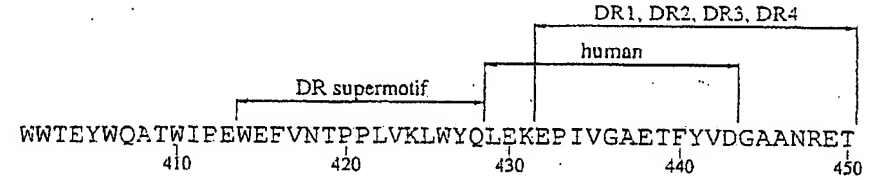


Figure 23: RT T-Helper Map aa 451–500

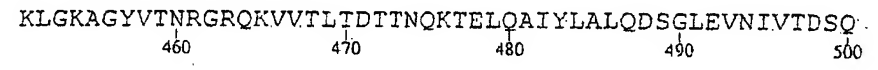
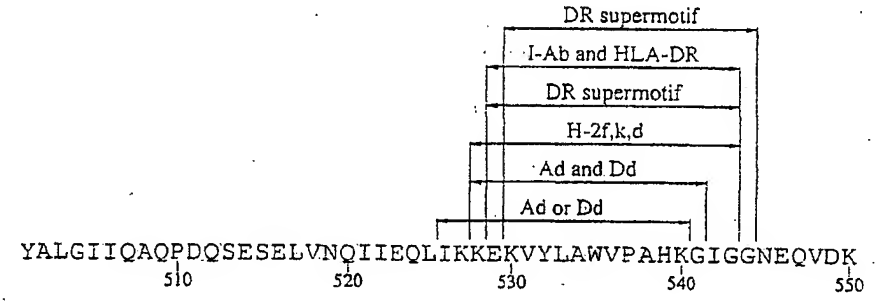


Figure 24: RT T-Helper Map aa 501–550



RT T-Helper Epitope Map

RT T-Helper Map aa 551–560

LVSAGIRKVL
560

13 Nef T-Helper Epitope Map

Nef T-Helper Map aa 1–50

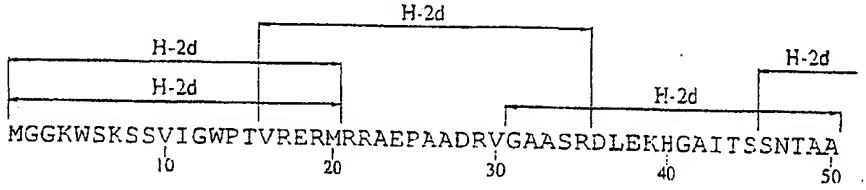


Figure 65: Nef T-Helper Map aa 51–100

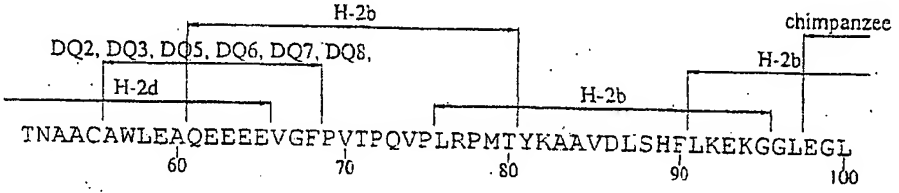


Figure 66: Nef T-Helper Map aa 101–150

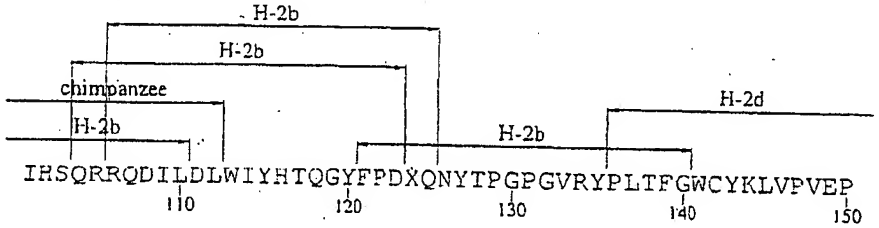


Figure 67: Nef T-Helper Map aa 151–200

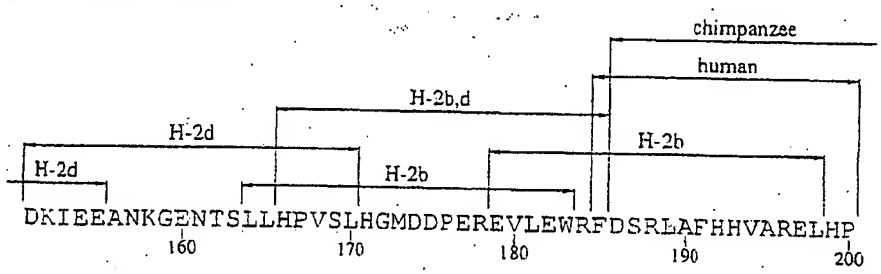
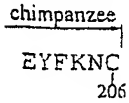


Figure 68: Nef T-Helper Map aa 201–206



CLAIMS

1. A recombinant polypeptide comprising amino acid sequence derived from

(i) an HIV gag gene product;

(ii) an HIV pol gene product; and

(iii) an HIV nef gene product,

said sequence being mutated with respect to the natural sequence of said gene product, and said sequence maintaining substantially all of the naturally occurring CD8+ T cell epitopes of said gene product as defined in p17 and p24 (gag), amino acids 1-440 of RT (pol) and nef shown in Example 8, said polypeptide comprising amino acid sequence having at least 75% identity to SEQ ID NO:9.

2. A recombinant polypeptide according to claim 1 wherein said polypeptide comprises amino acid sequence having at least 95% identity to SEQ ID NO:9.

3. A recombinant polypeptide according to claim 1 or claim 2 wherein the sequence identity is interepitope sequence identity.

4. A recombinant polypeptide according to claim 3 wherein said polypeptide comprises SEQ ID NO:9.

5. A recombinant polypeptide according to any of claims 1 to 4 wherein substantially all of the naturally occurring T helper epitopes of said gene product as defined in p17 and p24 (gag), amino acids 1-440 of RT (pol) and nef shown in Example 8 are maintained.

6. A recombinant polypeptide comprising amino acid sequence derived from at least two of

(i) an HIV gag gene product;

(ii) an HIV pol gene product; or

(iii) an HIV nef gene product,

said sequence being mutated with respect to the natural sequence of said gene product, and said sequence maintaining substantially all of the naturally occurring CD8+ T cell epitopes of the corresponding part(s) of said gene product as defined in p17 and p24 (gag), amino acids 1-440 of RT (pol) and nef shown in Example 8.

7. A recombinant polypeptide according to claim 6 comprising amino acid sequence derived from (i) and (ii) and (iii).

8. A recombinant polypeptide according to any preceding claim, said polypeptide comprising amino acid sequence derived from an HIV nef gene product, said recombinant polypeptide sequence being mutated to disrupt the function of said nef sequence, said nef sequence further comprising one or more T helper epitopes which are not present in the naturally occurring nef gene.

9. A recombinant polypeptide according to claim 8 comprising one or more T helper epitopes which are not present in the naturally occurring nef sequence and are shown in Figure 3A.

10. A recombinant polypeptide according to claim 8 or claim 9 comprising one or more T helper epitope(s) which are shown in Figure 3A and which are absent from Figure 3B.

11. A recombinant polypeptide according to any of claims 8 to 10 further comprising all naturally occurring CD8+ T cell epitopes of the nef gene product as defined in Example 8.

12. A recombinant polypeptide according to any of claims 8 to 11 further comprising all naturally occurring nef human T helper epitopes as defined in Example 8.

13. A recombinant polypeptide according to any of claims 8 to 12 wherein said polypeptide comprises sequence as shown in SEQ ID NO:6, or a sequence having at least 95% identity thereto.

14. A recombinant polypeptide according to any preceding claim, said polypeptide comprising amino acid sequence derived from an HIV pol gene product, said recombinant polypeptide sequence being mutated to disrupt the reverse transcriptase activity of the pol sequence, wherein substantially all of the CD8+ T cell epitopes of the naturally occurring pol sequence as defined in amino acids 1-440 of RT (pol) shown in Example 8 are retained in said recombinant polypeptide.

15. A recombinant polypeptide according to claim 14, wherein the reverse transcriptase activity of said pol sequence is mutated by duplication of an internal sequence derived from the centre of the naturally occurring pol gene and exchange of the amino and carboxy terminal portions of said pol sequence.

16. A recombinant polypeptide according to claim 15 wherein said duplicated internal sequence comprises TPDKKHQKEPPF (SEQ ID NO:4).

17. A recombinant polypeptide according to claim 15 or claim 16 wherein said polypeptide comprises sequence as shown in SEQ ID NO:12 or a sequence having at least 95% identity thereto.

18. A recombinant polypeptide according to any preceding claim, said polypeptide comprising amino acid sequence derived from an HIV gag gene product, said recombinant polypeptide sequence being mutated to disrupt processing of the gag gene product, and said gag sequence further comprising a disrupted myristoylation site, wherein substantially all of the CD8+ T cell epitopes of the naturally occurring gag sequence as defined in p17 and p24 (gag) shown in Example 8 are retained in said recombinant polypeptide.

19. A recombinant polypeptide according to claim 18 wherein the processing of gag is disrupted by exchanging the p17 and p24 domains and wherein the myristoylation site is disrupted by mutation of the second glycine to alanine.

20. A recombinant polypeptide according to claim 18 or claim 19 wherein said polypeptide comprises sequence as shown in SEQ ID NO:13 or a sequence having at least 95% identity thereto.

21. A recombinant polypeptide according to any preceding claim further comprising an antibody recognition tag wherein said tag is an HA tag comprising the sequence as shown in SEQ ID NO:8.

22. A recombinant polypeptide according to any preceding claim further comprising a CD8+ T cell epitope tag wherein said tag is a gp160 derived tag comprising the sequence as shown in SEQ ID NO:7.

23. A recombinant polypeptide according to any preceding claim, said polypeptide comprising the sequence as shown in SEQ ID NO:1.

24. A recombinant polypeptide according to any preceding claim wherein the HIV is a clade B HIV.

25. A recombinant nucleic acid encoding a polypeptide according to any preceding claim.

26. A recombinant nucleic acid sequence comprising SEQ ID NO:11, or a sequence which differs only by silent mutations with respect to the genetic code, or a sequence having at least 95% identity thereto.

27. A viral vector encoding a polypeptide according to any one of claims 1 to 24, said viral vector optionally being selected from the group consisting of poxviruses, adenoviruses, AAV, alphavirus, VSV, HSV and Sendai virus.

28. A viral vector according to claim 27 wherein said vector is an MVA or MVA derived vector.

29. A viral vector according to claim 27 wherein said vector is a fowlpox or fowlpox derived vector.

30. A viral vector according to claim 29 wherein said vector is an FP9 fowlpox vector.

31. A nucleic acid vector comprising a nucleic acid sequence according to claim 25 or claim 26 or encoding a polypeptide according to any one of claims 1 to 24.

32. An adenovirus vector comprising a nucleic acid sequence according to claim 25 or claim 26 or encoding a polypeptide according to any one of claims 1 to 24.

33. A poxvirus vector comprising a nucleic acid sequence according to claim 25 or claim 26 or encoding a polypeptide according to any one of claims 1 to 24.

34. A plasmid selected from the group consisting of p29D.gpn, pOPK6.gpn and pSG2.gpn.

35. Use of a polypeptide according to any one of claims 1 to 24 in medicine.

36. Use of polypeptide according to any one of claims 1 to 24 in the preparation of a medicament for the treatment or prevention of HIV infection.

37. Use of polypeptide according to any one of claims 1 to 24 in the preparation of a medicament for immunisation against HIV infection.

38. Use of a nucleic acid or a vector according to any one of claims 25 to 34 in medicine.

39. Use of a nucleic acid or a vector according to any one of claims 25 to 34 in the preparation of a medicament for the treatment or prevention of HIV infection.

40. Use of a nucleic acid or a vector according to any one of claims 25 to 34 in the preparation of a medicament for immunisation against HIV infection.

41. A method of immunising a subject against HIV infection comprising administering to said subject a polypeptide according to any one of claims 1 to 24 or a nucleic acid or vector according to any one of claims 25 to 34.

42. Use of a polypeptide according to any one of claims 1 to 24 or a nucleic acid or a vector according to any of claims 25 to 34 as a priming agent or as a boosting agent in a prime-boost immunisation regime.

43. Use of a polypeptide according to any one of claims 1 to 24 or a nucleic acid or a vector according to any of claims 25 to 34 in the induction of an immune response.

44. Use according to claim 43, wherein the immune response is selected from the group consisting of a CD8+ T cell response, a CD4+ T cell response, and a humoral response.

45. A method for inducing an immune response in a subject, comprising administering to said subject a polypeptide according to any one of claims 1 to 24 or a nucleic acid or a vector according to any of claims 25 to 34.

46. A method according to claim 45, wherein the immune response is selected from the group consisting of a CD8+ T cell response, a CD4+ T cell response, and a humoral response.

47. A recombinant polypeptide comprising amino acid sequence having at least 95% identity to the amino acid sequence presented in SEQ ID NO:9.

48. A Herpes Simplex Virus (HSV) vector comprising a nucleic acid sequence according to claim 25 or claim 26 or encoding a polypeptide according to any one of claims 1 to 24.

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49. A recombinant polypeptide, recombinant polynucleotide or viral vector substantially as hereinbefore described with reference to the accompanying drawings.

AMENDED CLAIMS

[received by the International Bureau on 28 February 2005 (28.02.05);
original claims 1-49 replaced by new claims 1-56 (7 pages)]

1. A recombinant polypeptide comprising amino acid sequence derived from

(i) an HIV gag gene product;
(ii) an HIV pol gene product; and
(iii) an HIV nef gene product,

said sequence being mutated with respect to the natural sequence of said gene products, and said sequence maintaining substantially all of the naturally occurring CD8+ T cell epitopes of said gene products, said polypeptide comprising an amino acid sequence having at least 75% identity to SEQ ID NO:9.

2. A recombinant polypeptide according to claim 1 wherein the naturally occurring CD8+ T cell epitopes of said gene products are as defined in p17 and p24 (gag), amino acids 1-440 of RT (pol) and nef shown in Example 8.

3. A recombinant polypeptide according to claim 1 wherein said polypeptide comprises an amino acid sequence having at least 95% identity to SEQ ID NO:9.

4. A recombinant polypeptide according to any preceding claim wherein the sequence identity is interepitope sequence identity.

5. A recombinant polypeptide according to claim 4 wherein said polypeptide comprises SEQ ID NO:9.

6. A recombinant polypeptide according to any preceding claim wherein substantially all of the naturally occurring T helper epitopes of said gene products are maintained.

7. A recombinant polypeptide according to claim 6 where the naturally occurring T helper epitopes of said gene products are as defined in p17 and p24 (gag), amino acids 1-440 of RT (pol) and nef shown in Example 8

8. A recombinant polypeptide comprising amino acid sequence derived from at least two of

(i) an HIV gag gene product;

(ii) an HIV pol gene product; or

(iii) an HIV nef gene product,

said sequence being mutated with respect to the natural sequence of said gene products, and said sequence maintaining substantially all of the naturally occurring CD8+ T cell epitopes of the corresponding part(s) of said gene products.

9. A recombinant polypeptide according to claim 8 wherein the naturally occurring CD8+ T cell epitopes are as defined in p17 and p24 (gag), amino acids 1-440 of RT (pol) and nef shown in Example 8.

10. A recombinant polypeptide according to claim 8 comprising amino acid sequence derived from (i) and (ii) and (iii).

11. A recombinant polypeptide according to any preceding claim, said polypeptide comprising amino acid sequence derived from an HIV nef gene product, said recombinant polypeptide sequence being mutated to disrupt the function of said nef sequence, said nef sequence further comprising one or more T helper epitopes which are not present in the naturally occurring nef gene.

12. A recombinant polypeptide according to claim 11 wherein the one or more T helper epitopes which are not present in the naturally occurring nef sequence are shown in Figure 3A.

13. A recombinant polypeptide according to claim 11 or claim 12 wherein the one or more T helper epitope(s) which are not present in the naturally occurring nef sequence are shown in Figure 3A and are absent from Figure 3B.

14. A recombinant polypeptide according to any of claims 11 to 13 further comprising all naturally occurring CD8+ T cell epitopes of the nef gene product.

15. A recombinant polypeptide according to claim 14 wherein the naturally occurring CD8+ T cell epitopes of the nef gene product are as defined in Example 8

16. A recombinant polypeptide according to any of claims 11 to 15 further comprising all naturally occurring nef human T helper epitopes.

17. A recombinant polypeptide according to claim 16 wherein the naturally occurring nef human T helper epitopes are as defined in Example 8.

18. A recombinant polypeptide according to any of claims 11 to 17 wherein said polypeptide comprises an amino acid sequence as shown in SEQ ID NO:6, or a sequence having at least 95% identity thereto.

19. A recombinant polypeptide according to any preceding claim, said polypeptide comprising amino acid sequence derived from an HIV pol gene product, said recombinant polypeptide sequence being mutated to disrupt the reverse transcriptase activity of the pol sequence, wherein substantially all of the CD8+ T cell epitopes of the naturally occurring pol sequence are retained in said recombinant polypeptide.

20. A recombinant polypeptide according to claim 19 wherein the CD8+ T cell epitopes of the naturally occurring pol sequence are as defined in amino acids 1-440 of RT (pol) shown in Example 8.

21. A recombinant polypeptide according to claim 19 or 20 , wherein the reverse transcriptase activity of said pol sequence is mutated by duplication of an internal

sequence derived from the centre of the naturally occurring pol gene and exchange of the amino and carboxy terminal portions of said pol sequence.

22. A recombinant polypeptide according to claim 21 wherein said duplicated internal sequence comprises TPDKKHQKEPPF (SEQ ID NO:4).

23. A recombinant polypeptide according to claim 21 or claim 22 wherein said polypeptide comprises an amino acid sequence as shown in SEQ ID NO:12 or a sequence having at least 95% identity thereto.

24. A recombinant polypeptide according to any preceding claim, said polypeptide comprising amino acid sequence derived from an HIV gag gene product, said recombinant polypeptide sequence being mutated to disrupt processing of the gag gene product, and said gag sequence further comprising a disrupted myristoylation site, wherein substantially all of the CD8+ T cell epitopes of the naturally occurring gag sequence are retained in said recombinant polypeptide.

25. A recombinant polypeptide according to claim 24 wherein the CD8+ T cell epitopes of the naturally occurring gag sequence are as defined in p17 and p24 (gag) shown in Example 8.

26. A recombinant polypeptide according to claim 24 or 25 wherein the processing of gag is disrupted by exchanging the p17 and p24 domains and wherein the myristoylation site is disrupted by mutation of the second glycine to alanine.

27. A recombinant polypeptide according to any one of claims 24-26 wherein said polypeptide comprises an amino acid sequence as shown in SEQ ID NO:13 or a sequence having at least 95% identity thereto.

28. A recombinant polypeptide according to any preceding claim further comprising an antibody recognition tag wherein said tag is an HA tag comprising the sequence as shown in SEQ ID NO:8.

29. A recombinant polypeptide according to any preceding claim further comprising a CD8+ T cell epitope tag wherein said tag is a gp160 derived tag comprising the sequence as shown in SEQ ID NO:7.

30. A recombinant polypeptide according to any preceding claim, said polypeptide comprising the sequence as shown in SEQ ID NO:1.

31. A recombinant polypeptide according to any preceding claim wherein the HIV is a clade B HIV.

32. A recombinant nucleic acid encoding a polypeptide according to any preceding claim.

33. A recombinant nucleic acid sequence comprising SEQ ID NO:11, or a sequence which differs only by silent mutations with respect to the genetic code, or a sequence having at least 95% identity thereto.

34. A viral vector encoding a polypeptide according to any one of claims 1 to 31, said viral vector optionally being selected from the group consisting of poxviruses, adenoviruses, AAV, alphavirus, VSV, HSV and Sendai virus.

35. A viral vector according to claim 33 wherein said vector is an MVA or MVA derived vector.

36. A viral vector according to claim 34 wherein said vector is a fowlpox or fowlpox derived vector.

37. A viral vector according to claim 36 wherein said vector is an FP9 fowlpox vector.

38. A nucleic acid vector comprising a nucleic acid sequence according to claim 32 or claim 33 or encoding a polypeptide according to any one of claims 1 to 31.

39. An adenovirus vector comprising a nucleic acid sequence according to claim 32 or claim 33 or encoding a polypeptide according to any one of claims 1 to 31.

40. A poxvirus vector comprising a nucleic acid sequence according to claim 32 or claim 33 or encoding a polypeptide according to any one of claims 1 to 31.

41. A plasmid selected from the group consisting of p29D.gpn, pOPK6.gpn and pSG2.gpn.

42. Use of a polypeptide according to any one of claims 1 to 31 in medicine.

43. Use of polypeptide according to any one of claims 1 to 31 in the preparation of a medicament for the treatment or prevention of HIV infection.

44. Use of polypeptide according to any one of claims 1 to 31 in the preparation of a medicament for immunisation against HIV infection.

45. Use of a nucleic acid or a vector according to any one of claims 32 to 41 in medicine.

46. Use of a nucleic acid or a vector according to any one of claims 32 to 41 in the preparation of a medicament for the treatment or prevention of HIV infection.

47. Use of a nucleic acid or a vector according to any one of claims 32 to 41 in the preparation of a medicament for immunisation against HIV infection.

48. A method of immunising a subject against HIV infection comprising administering to said subject a polypeptide according to any one of claims 1 to 31 or a nucleic acid or vector according to any one of claims 32 to 41.

49. Use of a polypeptide according to any one of claims 1 to 31 or a nucleic acid or a vector according to any of claims 32 to 41 as a priming agent or as a boosting agent in a prime-boost immunisation regime.

50. Use of a polypeptide according to any one of claims 1 to 31 or a nucleic acid or a vector according to any of claims 32 to 41 in the induction of an immune response.

51. Use according to claim 50, wherein the immune response is selected from the group consisting of a CD8+ T cell response, a CD4+ T cell response, and a humoral response.

52. A method for inducing an immune response in a subject, comprising administering to said subject a polypeptide according to any one of claims 1 to 31 or a nucleic acid or a vector according to any of claims 32 to 41.

53. A method according to claim 52, wherein the immune response is selected from the group consisting of a CD8+ T cell response, a CD4+ T cell response, and a humoral response.

54. A recombinant polypeptide comprising an amino acid sequence having at least 95% identity to the amino acid sequence presented in SEQ ID NO:9.

55. A Herpes Simplex Virus (HSV) vector comprising a nucleic acid sequence according to claim 32 or claim 33 or encoding a polypeptide according to any one of claims 1 to 31.

56. A recombinant polypeptide, recombinant polynucleotide or viral vector substantially as hereinbefore described with reference to the accompanying drawings.

STATEMENT

Statement Under Article 19(1)

PCT Patent Application No. PCT/GB2004/004038

The amended claim set finds basis in the application as originally filed, in particular in the claims of the application as originally filed. The amended claims do not add matter to the application.

Original claims 1, 5, 6, 11, 12 and 18 have each been divided into amended claims 1 and 2, 6 and 7, 8 and 9, 14 and 15, 16 and 17, 24 and 25 in order to improve clarity.

Original claims 2, 13, 17, 20 and 47 have been amended into new claims 3, 18, 23, 27 and 54 by adding "an" or "an amino acid".

Original claims 9 and 10 have been amended into new claims 12 and 13 in order to improve the references to figures 3a and 3b.

The remaining claims have been amended purely to maintain the correct dependency.

FIG. 1

GAG p24 M A P I V Q N L Q G Q M V H Q A I S P R T L N A
W V K V V E E K A F S P E V I P M F S A L S E G A T P
Q D L N T M L N T V G G H Q A A M Q M L K E T I N E E
A A E W D R L H P V H A G P I A P G Q M R E P R G S D
I A G T T S T L Q E Q I G W M T N N P P I P V G E I Y
K R W I I L G L N K I V R M Y S P T S I L D I R Q G P
K E P F R D Y V D R F Y K T L R A E Q A S Q E V K N W
M T E T L L V Q N A N P D C K T I L K A L G P A A T L
E E M M T A C Q G V G G P G H K A R V L

GAG p17 M A A R A S V L S G G E L D R W E K I R L R P G
G K K K Y K L K H I V W A S R E L E R F A V N P G L L
E T S E G C R Q I L G Q L Q P S L Q T G S E E L R S L
Y N T V A T L Y C V H Q R I E V K D T K E A L E K I E
E E Q N K S K K K A Q Q A A A D T G N S S Q V S Q N Y

POL Reverse Transcriptase duplicated sequence T P D K K H Q K E P P F

POL Reverse Transcriptase L W M G Y E L H P D K W T V Q P I V
L P E K D S W T V N D I Q K L V G K L N W A S Q I Y A
G I K V K Q L C K L L R G T K A L T E V I P L T E E A
E L E L A E N R E I L K E P V H G V Y Y D P S K D L I
A E I Q K Q G Q G Q W T Y Q I Y Q E P F K N L K T G K
Y A R M R G A H T N D V K Q L T E A V Q K I A T E S I
V I W G K T P K F K L P I Q K E T W E A W W T E Y W Q
A T W I P E W E F V N T P P L V K L W Y Q L E K E P I
V G A E T F P I S P I E T V P V K L K P G M D G P K V
K Q W P L T E E K I K A L V E I C T E M E K E G K I S
K I G P E N P Y N T P V F A I K K K D S T K W R K L V
D F R E L N K R T Q D F W E V Q L G I P H P A G L K K
K K S V T V L D V G D A Y F S V P L D K D F R K Y T A
F T I P S I N N E T P G I R Y Q Y N V L P Q G W K G S
P A I F Q S S M T K I L E P F R K Q N P D I V I Y Q Y
M D D L Y V G S D L E I G Q H R T K I E E L R Q H L L
R W G F T

POL Reverse Transcriptase duplicated sequence T P D K K H Q K E P P F

Scrambled NEF L V W K F D S R L A F H H M A R E L H P E Y
Y K D C D P E K E V L V W K F D A N E G E N N S L L H
P M S L H G M D D P E K E V P E K V E E A N E G E N G
P G I R Y P L T F G W C F K L V P V E P E K V E E W Q
N Y T P G P G I R Y Q K R Q D I L D L W V Y H T Q G Y
F P D W Q N Y T P E G L I Y S Q K R Q D I P M T Y K A
A L D L S H F L K E K G G L E G L I Y S P Q V P L R P
M T Y K A A D C A W L E A Q E E E E V G F P V R P Q V
P L R N T A A N N A D C A W L A D G V G A V S R D L E
K H G A I T S S N T A A N N R R A E P A A D G V G A M
G G K W S K R S V V G W P T V R E R M R R A E P A

gp160 murine CD8+ tag R G P G R A F V T I

HA tag Y P Y D V P D Y A

FIG. 2

Scrambled NEF from gpn

LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEV
PEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTPGPGIRYQKRQDILDLWVYH
TQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKA
ADCAWLEAQEEEEVGFPVRPQVPLRNTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANN
RRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA

Native Nef

M	G	G	K	W	S	K	R	S	V	V	G	W	P	T	V	R	E	R	M	R	R	A	E	P	A	A	D	G	V	G	A
V	S	R	D	L	E	K	H	G	A	I	T	S	S	N	T	A	A	N	N	A	D	C	A	W	L	E	A	Q	E	E	E
E	V	G	F	P	V	R	P	Q	V	P	L	R	P	M	T	Y	K	A	A	L	D	L	S	H	F	L	K	E	K	G	G
L	E	G	L	I	Y	S	Q	K	R	Q	D	I	L	D	L	W	V	Y	H	T	Q	G	Y	F	P	D	W	Q	N	Y	T
P	G	P	G	I	R	Y	P	L	T	F	G	W	C	E	K	L	V	P	V	E	P	E	K	V	E	E	A	N	E	G	E
N	N	S	L	L	H	P	M	S	L	H	G	M	D	D	P	E	K	E	V	L	V	W	K	F	D	S	R	L	A	F	H
H	M	A	R	E	L	H	P	E	Y	Y	K	D	C																		

FIG. 3A

MHC Class-II Binding Prediction Results for Scrambled nef using ProPred

INPUT & PARAMETER INFORMATION

Antigen Name	Scrambled Nef
Scanned on	Tue Aug 26 03:13:51 2003
Length of input sequence	290 amino acids
Number of nanomers from input sequence	282
Number of nanomers with obligatory P1 anchor residue	83
Threshold setting	3
Number of alleles in query	51
Predicted epitopes	underlined
Predicted anchor residues	*italicized*

---------10---------20---------30---------40---------50---------60---------70---------80---------90---------100-----
--110-------120-------130-------140-------150-------160-------170-------180-------190-------200-------210--
-----220-------230-------240-------250-------260-------270-------280-------

DRB1_0101:

LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA

DRB1_0102:

LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA

DRB1_0301:

LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA

DRB1_0305:

LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA

FIG. 3A CONT'D

DRB1_0306:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0307:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0308:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0309:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0311:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0401:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0402:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0404:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0405:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA

FIG. 3A CONT'D

DRB1_0408:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0410:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0421:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0423:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0426:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0701:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0703:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0801:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0802:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA

FIG. 3A CONT'D

DRB1_0804:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0806:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0813:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_0817:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1101:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1102:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1104:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1106:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1107:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA

FIG. 3A CONT'D

DRB1_1114:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1120:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1121:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1128:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1301:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1302:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1304:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1305:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1307:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1311:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA

FIG. 3A CONT'D

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DRB1_1321:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1322:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1323:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1327:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1328:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1501:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1502:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB1_1506:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB5_0101:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
DRB5_0105:
LVWKFDSRLAFHHMARELHPEYYKDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDDPEKEVPEKVEEANEGENGPGIRYPLTFGWCEKLVPVEPEKVEEWQNYTP
GPGIRYQKRQDILDLWVYHTQGYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSHFLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQEEEEVGFPVRPQVPLR
NTAANNADCAWLADGVGAVSRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGKWSKRSVVGWPTVRERMRRAEPA
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FIG. 3B

MHC Class-II Binding Prediction Results for native nef using ProPred

INPUT & PARAMETER INFORMATION

Antigen Name	Nef
Scanned on	Tue Aug 26 03:11:18 2003
Length of input sequence	206 amino acids
Number of nanomers from input sequence	198
Number of nanomers with obligatory P1 anchor residue	61
Threshold setting	3
Number of alleles in query	51
Predicted epitopes	underlined
Predicted anchor residues	*italicized*

---------10--------20--------30--------40--------50--------60--------70--------80--------90--------100-------
--110-------120-------130-------140-------150-------160-------170-------180-------190-------200----

DRB1_0101:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC

DRB1_0102:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC

DRB1_0301:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC

DRB1_0305:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC

DRB1_0306:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC

FIG. 3B CONT'D

DRB1_0307:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0308:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0309:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0311:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0401:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0402:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0404:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0405:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0408:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0410:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0421:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0423:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC

FIG. 3B CONT'D

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DRB1_0426:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0701:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0703:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEEEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0801:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0802:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0804:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0806:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0813:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_0817:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1101:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1102:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1104:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
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FIG. 3B CONT'D

DRB1_1106:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1107:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1114:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1120:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1121:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1128:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1301:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1302:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1304:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1305:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1307:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1311:
MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC

FIG. 3B CONT'D

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DRB1_1321:
MGGKWSKRSVVGWPTVRERMRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1322:
MGGKWSKRSVVGWPTVRERMRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1323:
MGGKWSKRSVVGWPTVRERMRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1327:
MGGKWSKRSVVGWPTVRERMRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1328:
MGGKWSKRSVVGWPTVRERMRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1501:
MGGKWSKRSVVGWPTVRERMRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1502:
MGGKWSKRSVVGWPTVRERMRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB1_1506:
MGGKWSKRSVVGWPTVRERMRAEPAADGVGAVSRDLEKHGAITSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDLSHFLKEKGGLEGLIYSQKRQ
DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB5_0101:
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DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
DRB5_0105:
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DILDLWVYHTQGYFPDWQNYTPGPGIRYPLTFGWCEKLVPVEPEKVEEANEGENNSLLHPMSLHGMDDPEKEVLVWKFDSRLAFHHMARELHPEYYKDC
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FIG. 4

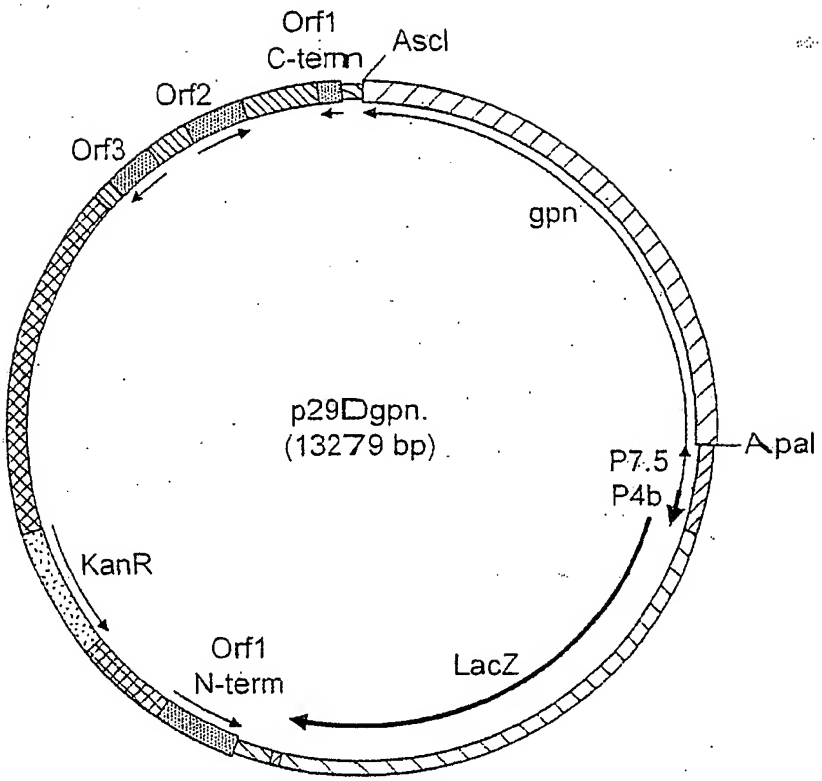

Orf1
C-term
AscI
Orf2
Orf3
gpn
p29Dgpn.
(13279 bp)
ApaI
P7.5
P4b
KanR
Orf1
N-term
LacZ

FIG. 5

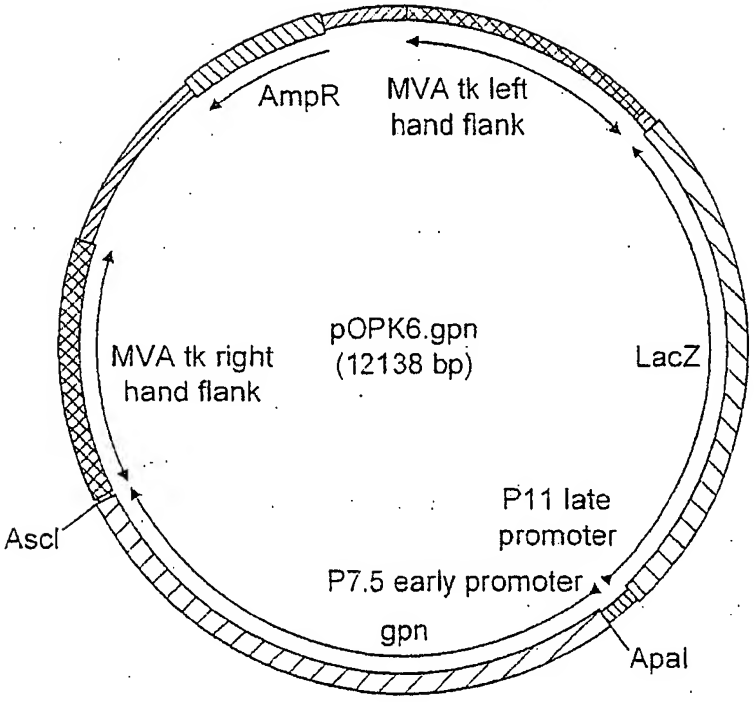

AmpR
MVA tk left hand flank
pOPK6.gpn (12138 bp)
MVA tk right hand flank
LacZ
AscI
P11 late promoter
P7.5 early promoter
gpn
ApaI

FIG. 6

gpn base sequence

CCGGGCCCGCCGCCACCATGGCCCCCATCGTGCAGAACCTGCAAGGCCAGATGGTGCACCAGGC
CATCAGCCCCAGAACCCTGAATGCCTGGGTGAAGGTGGTGGAGGAGAAGGCCTTCAGCCCTGAG
GTGATCCCTATGTTCAGCGCCCTGAGCGAGGGCGCCACCCCCCAGGACCTGAACACCATGCTGA
ATACCGTGGGCGGCCATCAGGCCGCCATGCAGATGCTGAAGGAGACCATCAACGAGGAGGCCGC
CGAGTGGGACAGACTGCACCCCGTGCACGCCGGACCTATCGCCCCTGGCCAGATGAAGAGAGCCC
AGAGGCAGCGACATCGCCGGCACCACCAGCACCCTGCAAGAGCAGATCGGCTGGATTGACCAACA
ACCCCCCTATCCCTGTGGGCGAGATCTACAAGAGATGGATCATCCTGGGCCTGAACAAGATCGT
GAGAATGTACAGCCCTACCAGCATCCTGGACATCAGACAGGGACCCAAGGAGCCCTTCAGAGAC
TACGTGGACAGGTTCTACAAGACCCTGAGAGCCGAGCAGGCCAGCCAGGAAGTGAAGAACTGGA
TGACCGAGACCCTGCTGGTGCAGAACGCCAACCCCGACTGCAAGACCATCCTGAAGGCCCTGGG
ACCTGCCGCTACCCTGGAGGAGATGATGACCGCCTGCCAGGGCGTGGGCGGACCTGGCCACAAG
GCCAGAGTGCTCATGGCCGCCAGAGCCAGCGTGCTGAGCGGCGGAGAGCTGGATAGATGGGAGA
AGATCAGACTGAGACCTGGCGGCAAGAAGAAGTACAAGCTGAAGCACATCGTGTGGGCCTCTAG
GGAGCTGGAGAGATTCGCCGTGAATCCTGGCCTGCTGGAGACCAGCGAGGGCTGTAGACAGATC
CTGGGCCAGCTGCAACCCAGCCTGCAAACCGGCTCTGAGGAGCTGAGATCCCTGTACAACACCG
TGGCCACCCTGTACTGCGTGCACCAGAGAATCGAGGTGAAGGACACAAAGGAGGCCCTGGAGAA
GATCGAGGAGGAGCAGAACAAGTCCAAGAAGAAGGCCCAGCAGGCCGCTGCCGACACCGGCAAC
AGCAGCCAGGTGAGCCAGAACTACACCCCCGACAAGAAGCACCAGAAGGAGCCCCCTTTCCTGT
GGATGGGCTACGAGCTGCACCCCGATAAGTGGACCGTGCAGCCCATCGTGCTGCCTGAGAAGGA
TAGCTGGACCGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGAACTGGGCCAGCCAGATCTAC
GCCGGCATCAAGGTGAAGCAGCTGTGTAAGCTGCTGAGAGGCACCAAGGCCCTCACAGAAGTGA
TCCCCCTCACAGAGGAGGCCGAGCTGGAGCTGGCCGAGAACAGAGAGATCCTGAAAGAGCCCGT
GCACGGCGTGTACTACGACCCCAGCAAGGATCTCATCGCCGAGATTCAGAAGCAGGGCCAGGGC
CAGTGGACCTACCAGATCTACCAGGAGCCTTTCAAGAACCTGAAAACCGGCAAGTACGCCAGAA
TGAGAGGCGCTCACACCAACGACGTGAAGCAGTTGACCGAGGCCGTGCAGAAAATCGCCACCGA
GAGCATCGTGATCTGGGGCAAGACCCCCAAGTTCAAGCTGCCTATCCAGAAGGAGACCTGGGAG
GCCTGGTGGACCGAGTATTGGCAGGCCACCTGGATTCCTGAGTGGGAGTTCGTGAACACCCCCC
CTCTGGTGAAGCTGTGGTATCAGCTGGAGAAGGAGCCTATCGTGGGCGCCGAGACCTTCCCCAT
CAGCCCTATCGAGACCGTGCCCGTGAAGCTGAAGCCCGGCATGGACGGCCCCAAGGTGAAACAG
TGGCCTCTCACCGAGGAGAAGATTAAGGCCCTGGTGGAGATTTGCACCGAGATGGAAGAAGGAAG
GCAAGATCAGCAAGATCGGCCCCGAGAATCCCTACAATACCCCCGTGTTCGCCATTAAGAAGAA
GGACTCCACCAAGTGGAGAAAGCTGGTGGACTTCAGAGAGCTGAACAAGAGAACCCAGGACTTC
TGGGAGGTGCAGCTGGGCATCCCCCACCCTGCCGGCCTGAAGAAGAAGAAGAGCGTGACCGTGC
TGGACGTGGGCGACGCCTACTTCAGCGTGCCCCTGGACAAGGACTTCAGAAAGTACACCGCCTT
CACCATCCCCAGCATCAACAACGAGACCCCCGGCATCAGATACCAGTACAACGTGCTGCCCCAG
GGCTGGAAGGGCAGCCCCGCCATCTTCCAGAGCAGCATGACAAAGATCCTGGAGCCCTTCCGGA
AGCAGAACCCCGATATCGTGATCTACCAGTACATGGACGACCTGTACGTGGGCAGCGATCTGGA
GATCGGCCAGCACAGAACCAAGATCGAAGAGCTGAGACAGCACCTGCTGAGATGGGGCTTCACC
ACACCAGATAAGAAACATCAGAAAGAACCACCATTCCTGGTGTGGAAGTTTGACAGCAGACTGG
CCTTCCACCATATGGCCAGAGAGCTGCATCCTGAGTACTACAAGGACTGCGATCCCGAGAAGGA
GGTGCTGGTCTGGAAATTCGACGCCAACGAGGGCGAGAACAACAGCCTGCTGCACCCCATGAGC
CTGCACGGCATGGATGATCCTGAGAAAGAAGTGCCCGAGAAGGTGGAAGAGGCCAATGAGGGAG
AAAACGGCCCTGGCATTAGATATCCCCTCACCTTCGGCTGGTGCTTCAAGCTGGTGCCTGTGGA
GCCTGAGAAAGTGGAGGAATGGCAGAATTACACACCCGGCCCAGGCATCCGGTATCAGAAGAGA
CAGGACATTCTGGACCTGTGGGTGTACCACACCCAGGGCTACTTCCCCGACTGGCAGAACTATA
CTCCTGAGGGCCTCATCTACAGCCAGAAGCGGCAGGATATCCCCATGACCTACAAGGCCGCCCT
GGATCTGAGCCACTTTCTGAAAGAGAAGGGCGGCCTGGAGGGCTTGATCTACTCCCCACAGGTG
CCACTGAGACCTATGACATATAAGGCTGCCGACTGCGCCTGGCTGGAGGCCCAGGAAGAGGAGG
AAGTGGGCTTCCCTGTGAGACCCCAGGTGCCCCTGCGGAACACCGCCGCCAATAATGCCGATTG
TGCTTGGCTCGCCGACGGCGTGGGAGCCGTGAGCAGaGACCTGGAAAAGCACGGCGCCATCACC
AGCAGCAATACAGCCGCCAACAACAGAAGAGCCGAGCCTGCCGCCGATGGAGTGGGCGCCATGG
GCGGCAAGTGGAGCAAGAGATCCGTGGTGGGCTGGCCCACCGTGAGAGAAAGAATGAGAAGGGC
CGAACCCGCCAGAGGACCCGGCAGAGCCTTCGTGACCATCTACCCCTACGACGTGCCCGACTAC
GCTTGATGAGGCGCGCCCT

FIG. 7

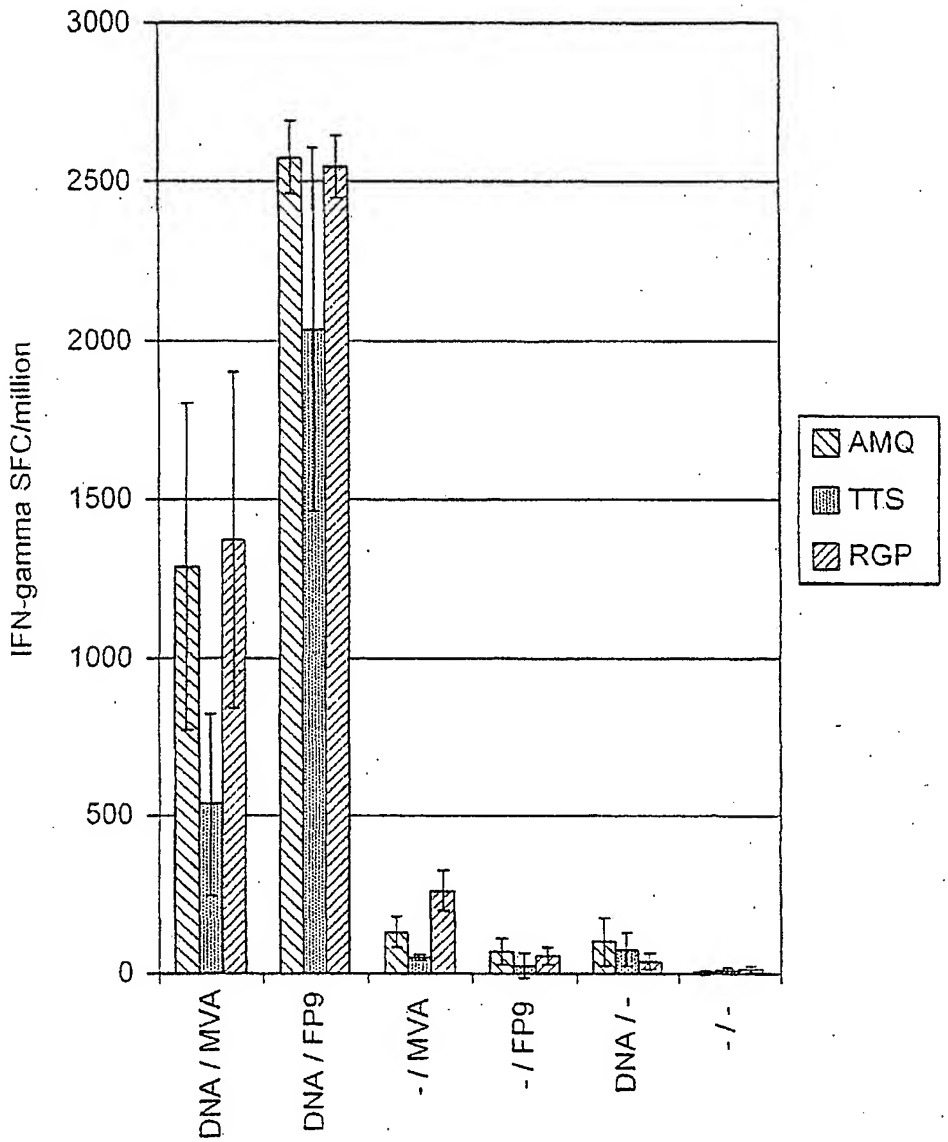

3000
2500
2000
1500
1000
500
0
IFN-gamma SFC/million
AMQ
TTS
RGP
DNA / MVA
DNA / FP9
- / MVA
- / FP9
DNA / -
- / -

FIG. 8

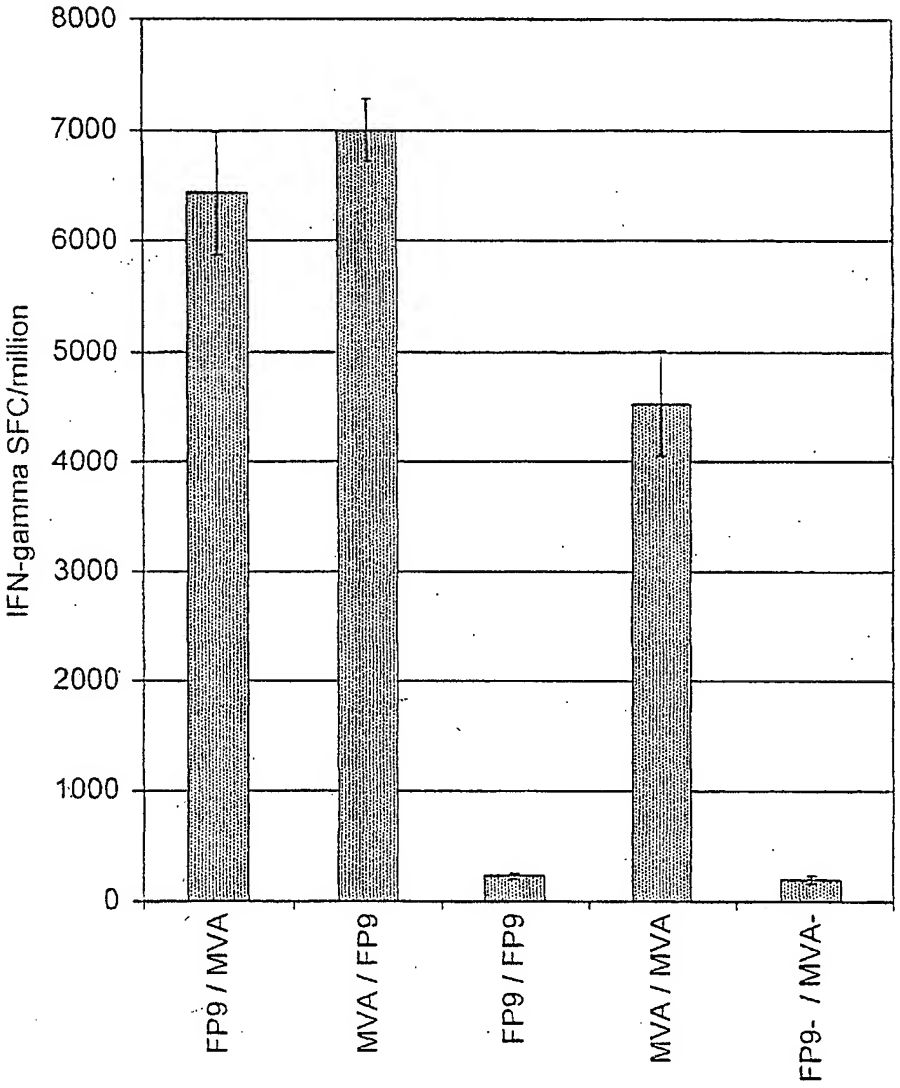

8000
7000
6000
5000
4000
3000
2000
1000
0
IFN-gamma SFC/million
FP9 / MVA
MVA / FP9
FP9 / FP9
MVA / MVA
FP9- / MVA-

FIG. 9

MAPIVQNLQGQMVHQAISPRTLNAWVKVVEEKAFSPEVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQMLKETINEEAAEWDRL
MAPIVQNLQGQMVHQAISPR (20)
GQMVHQAISPRTLNAWVKVV (20)
RTLNAWVKVVEEKAFSPEVI (20)
EEKAFSPEVIPMFSALSEGA (20)
PMFSALSEGATPQDLNTML (19)
ATPQDLNTMLNTVGGHQAAM (20)
NTVGGHQAAMQMLKETI (17)
AAMQMLKETINEEAAEWDRL (20)

FIG. 10

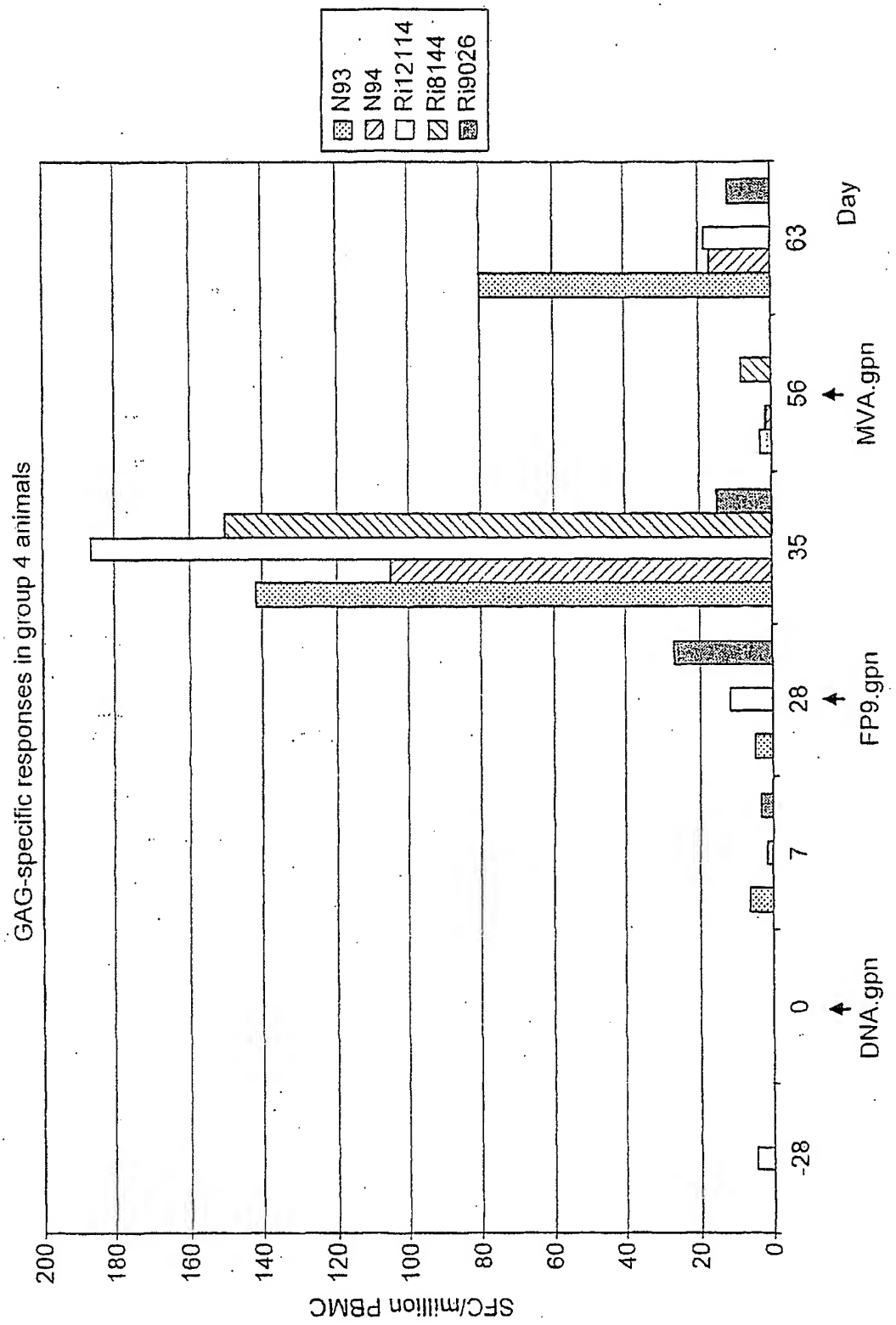

GAG-specific responses in group 4 animals
N93
N94
Ri12114
Ri8144
Ri9026
SFC/million PBMC
0
20
40
60
80
100
120
140
160
180
200
-28
0
7
28
35
56
63
Day
DNA.gpn
FP9.gpn
MVA.gpn

FIG. 11

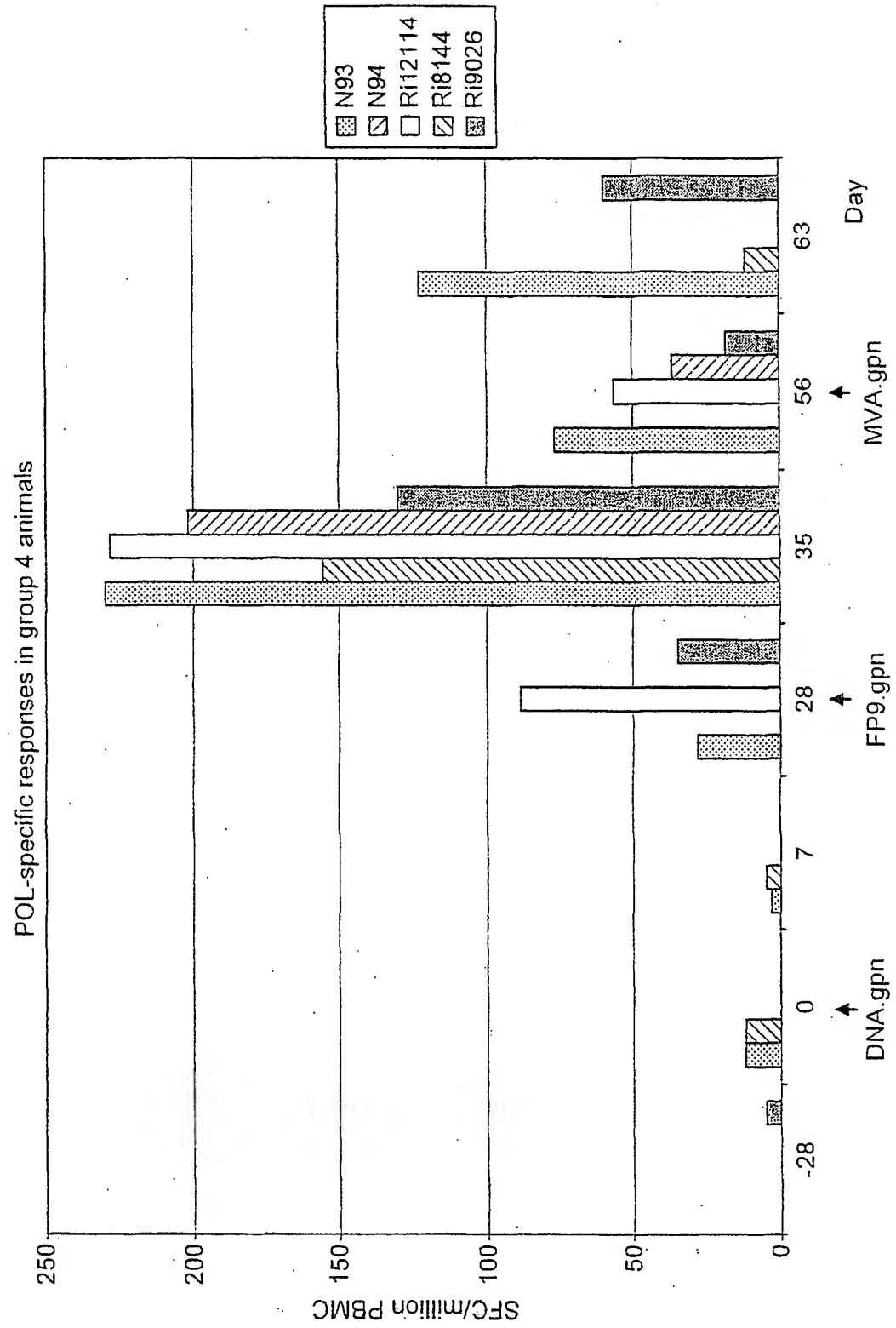

POL-specific responses in group 4 animals
N93
N94
Ri112114
Ri8144
Ri9026
SFC/million PBMC
250
200
150
100
50
0
-28
0
7
28
35
56
63
Day
DNA.gpn
FP9.gpn
MVA.gpn

FIG. 12

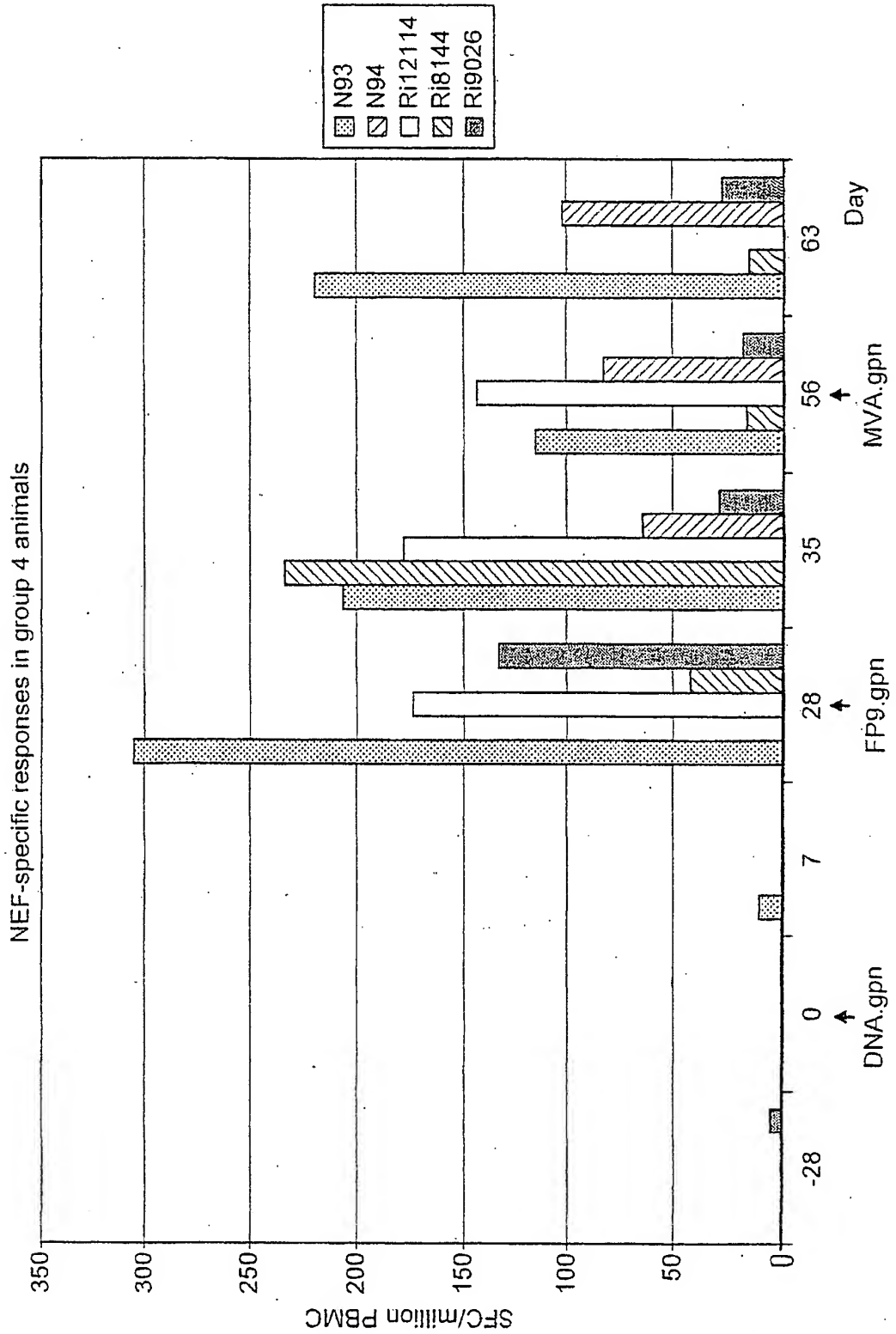

NEF-specific responses in group 4 animals
N93
N94
Ri12114
Ri8144
Ri9026
SFC/million PBMC
350
300
250
200
150
100
50
0
-28
0
7
28
35
56
63
Day
DNA.gpn
FP9.gpn
MVA.gpn

FIG. 13a

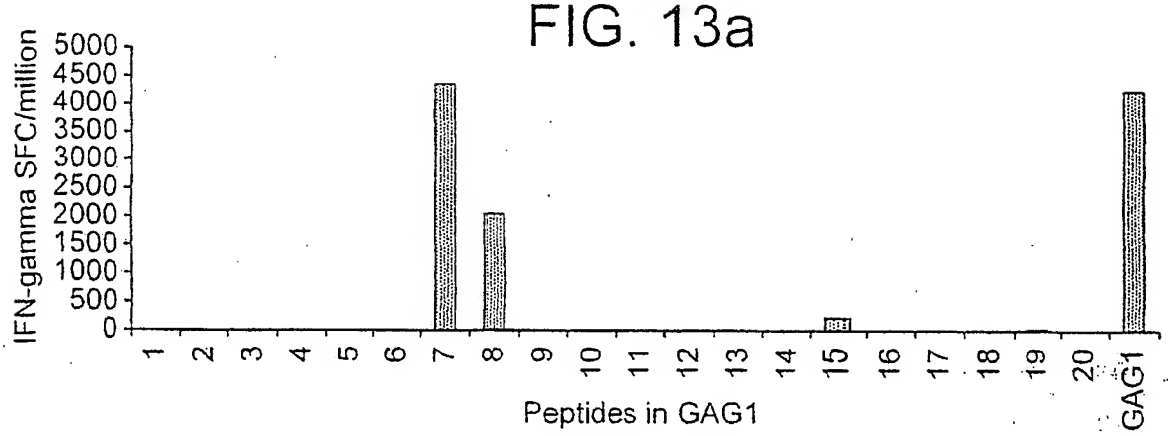


FIG. 13b

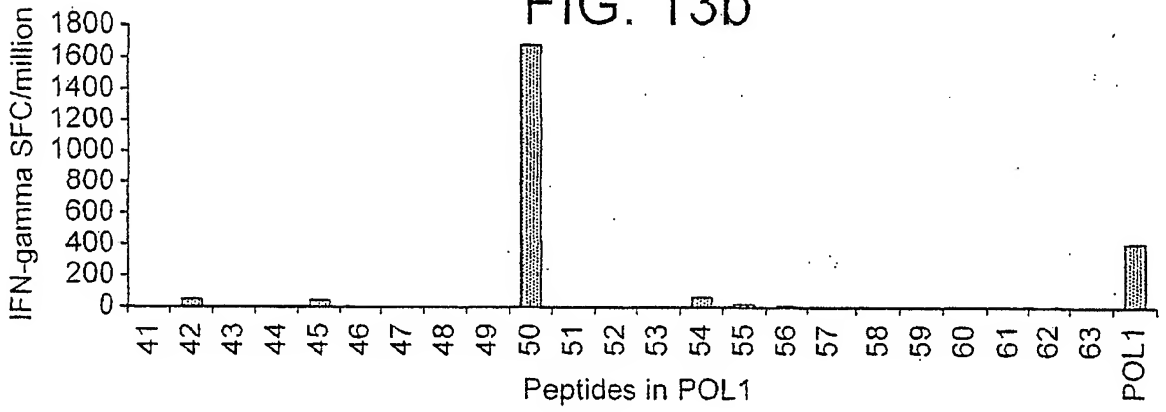


FIG. 13c

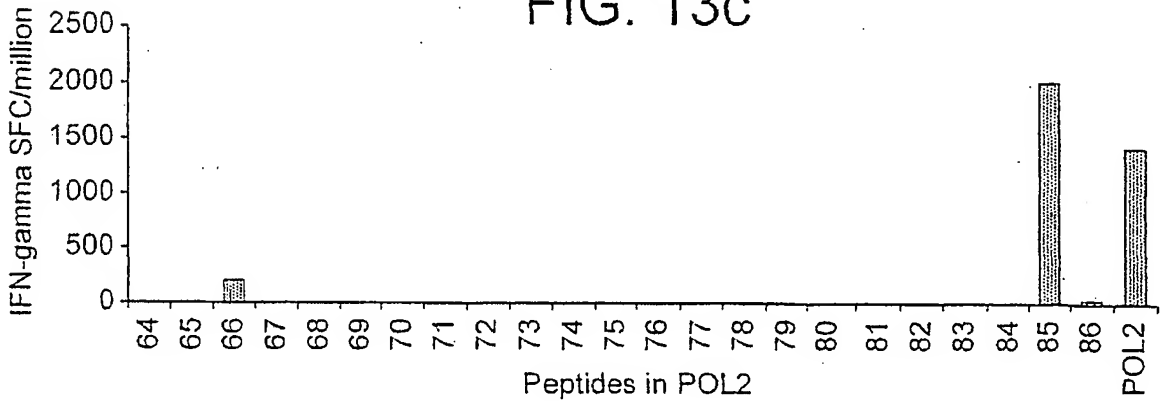


FIG. 13d

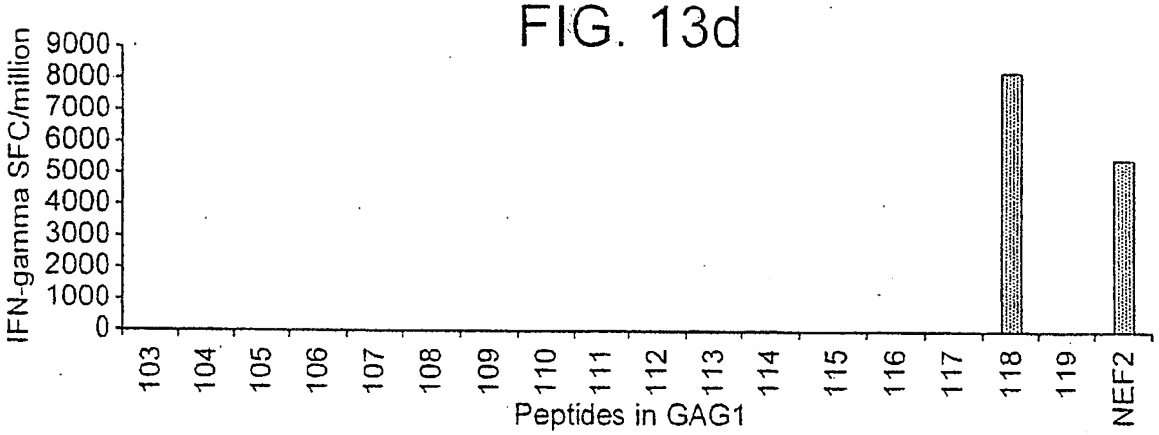
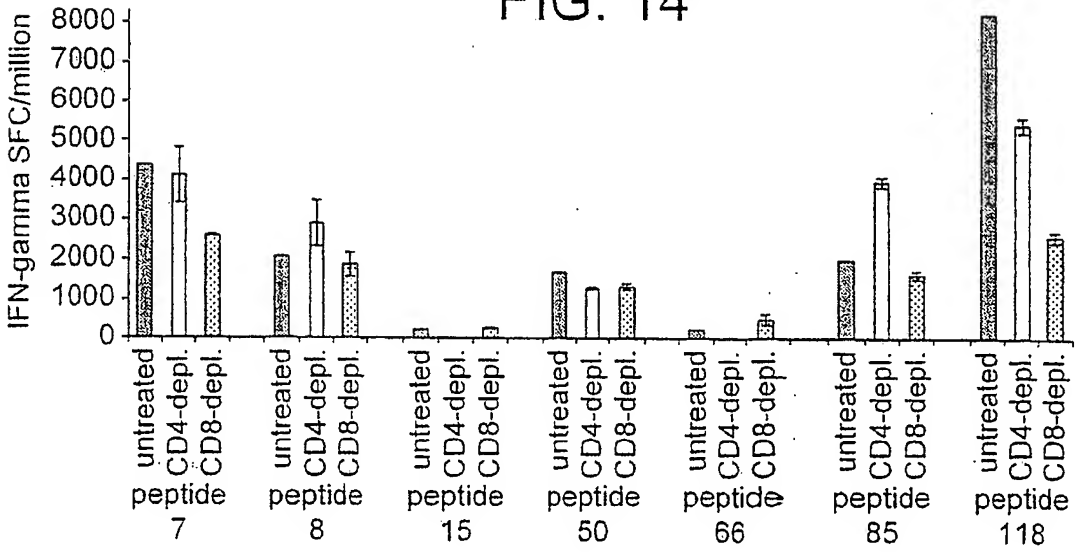


FIG. 14



Sequence Listing

SEQ ID NO:1 Artificial sequence – GPN

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LKETINEEAAEWDRLHPVHAGPIAPGQMREPRGSD
IAGTTSTLQEQIGWMTNNPPIPVGEIYKRWIILGL
NKIVRMYSPTSILDIRQGPKEPFRDYVDRFYKTLR
AEQASQEVKNWMTETLLVQNANPDCKTILKALGPA
ATLEEMMTACQGVGGPGHKARVLMAARASVLSGGE
LDRWEKIRLRPGGKKKYKLKHIVWASRELERFAVN
PGLLETSEGCRQILGQLQPSLQTGSEELRSLYNTV
ATLYCVHQRIEVKDTKEALEKIEEEQNKSKKKAQQ
AAADTGNSSQVSQNYTPDKKHQKEPPFLWMGYELH
PDKWTVQPIVLPEKDSWTVNDIQKLVGKLNWASQI
YAGIKVKQLCKLLRGTKALTEVIPLTEEAELELAE
NREILKEPVHGVYYDPSKDLIAEIQKQGQGQWTYQ
IYQEPFKNLKTGKYARMRGAHTNDVKQLTEAVQKI
ATESIVIWGKTPKFKLPIQKETWEAWWTEYWQATW
IPEWEFVNTPPLVKLWYQLEKEPIVGAETFPISPI
ETVPVKLKPGMDGPKVKQWPLTEEKIKALVEICTE
MEKEGKISKIGPENPYNTPVFAIKKKDSTKWRKLV
DFRELNKRTQDFWEVQLGIPHPAGLKKKKSVTVLD
VGDAYFSVPLDKDFRKYTAFTIPSINNETPGIRYQ
YNVLPQGWKGSPAIFQSSMTKILEPFRKQNPDIVI
YQYMDDLYVGSDLEIGQHRTKIEELRQHLLRWGFT
TPDKKHQKEPPFLVWKFDSRLAFHHMARELHPEYY
KDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDD
PEKEVPEKVEEANEGENGPGIRYPLTFGWCFKLVP
VEPEKVEEWQNYTPGPGIRYQKRQDILDLWVYHTQ
GYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSH
FLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQ
EEEEVGFPVRPQVPLRNTAANNADCAWLADGVGAV
SRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGK
WSKRSVVGWPTVRERMRRAEPARGPGRAFVTIYPY
DVPDYA

SEQ ID NO:2 Artificial sequence – GAG P24

MAPIVQNLQGQMVHQAISPRTLNAWVKVVEEKAFS
PEVIPMFSALSEGATPQDLNTMLNTVGGHQAAMQM
LKETINEEAAEWDRLHPVHAGPIAPGQMREPRGSD
IAGTTSTLQEQIGWMTNNPPIPVGEIYKRWIILGL
NKIVRMYSPTSILDIRQGPKEPFRDYVDRFYKTLR
AEQASQEVKNWMTETLLVQNANPDCKTILKALGPA
ATLEEMMTACQGVGGPGHKARVL

SEQ ID NO:3 Artificial sequence – GAG P17

MAARASVLSGGELDRWEKIRLRPGGKKKYKLKHIV
WASRELERFAVNPGLLETSEGCRQILGQLQPSLQT
GSEELRSLYNTVATLYCVHQRIEVKDTKEALEKIE
EEQNKSKKKAQQAAADTGNSSQVSQNY

SEQ ID NO:4 Artificial sequence – POL P51 duplication

TPDKKHQKEPPF

SEQ ID NO:5 Artificial sequence – POL P51 core

LWMGYELHPDKWTVQPIVLPEKDSWTVNDIQKLVG
KLNWASQIYAGIKVKQLCKLLRGTKALTEVIPLTE
EAELELAENREILKEPVHGVYYDPSKDLIAEIQKQ
GQGQWTYQIYQEPFKNLKTGKYARMRGAHTNDVKQ
LTEAVQKIATESIVIWGKTPKFKLPIQKETWEAWW
TEYWQATWIPEWEFVNTPPLVKLWYQLEKEPIVGA
ETFPISPIETVPVKLKPGMDGPKVKQWPLTEEKIK
ALVEICTEMEKEGKISKIGPENPYNTPVFAIKKKD
STKWRKLVDFRELNKRTQDFWEVQLGIPHPAGLKK
KKSVTVLDVGDAYFSVPLDKDFRKYTAFTIPSINN
ETPGIRYQYNVLPQGWKGSPAIFQSSMTKILEPFR
KQNPDIVIYQYMDDLYVGSDLEIGQHRTKIEELRQ
HLLRWGFT

SEQ ID NO:6 Artificial sequence – scrambled NEF

L V W K F D S R L A F H H M A R E L H P E Y Y K D C D P E K E V L V W
K F D A N E G E N N S L L H P M S L H G M D D P E K E V P E K V E E A
N E G E N G P G I R Y P L T F G W C F K L V P V E P E K V E E W Q N Y
T P G P G I R Y Q K R Q D I L D L W V Y H T Q G Y F P D W Q N Y T P E
G L I Y S Q K R Q D I P M T Y K A A L D L S H F L K E K G G L E G L I
Y S P Q V P L R P M T Y K A A D C A W L E A Q E E E E V G F P V R P Q
V P L R N T A A N N A D C A W L A D G V G A V S R D L E K H G A I T S
S N T A A N N R R A E P A A D G V G A M G G K W S K R S V V G W P T V
R E R M R R A E P A

SEQ ID NO:7 Artificial sequence – gp160 murine CD8 tag

R G P G R A F V T I

SEQ ID NO:8 Artificial sequence – HA-tag

Y P Y D V P D Y A

SEQ ID NO: 9 Artificial sequence - core GPN

M A P I V Q N L Q G Q M V H Q A I S P R T L N A W V K V V E E K A F S
P E V I P M F S A L S E G A T P Q D L N T M L N T V G G H Q A A M Q M
L K E T I N E E A A E W D R L H P V H A G P I A P G Q M R E P R G S D
I A G T T S T L Q E Q I G W M T N N P P I P V G E I Y K R W I I L G L
N K I V R M Y S P T S I L D I R Q G P K E P F R D Y V D R F Y K T L R
A E Q A S Q E V K N W M T E T L L V Q N A N P D C K T I L K A L G P A
A T L E E M M T A C Q G V G G P G H K A R V L M A A R A S V L S G G E
L D R W E K I R L R P G G K K K Y K L K H I V W A S R E L E R F A V N
P G L L E T S E G C R Q I L G Q L Q P S L Q T G S E E L R S L Y N T V
A T L Y C V H Q R I E V K D T K E A L E K I E E E Q N K S K K K A Q Q
A A A D T G N S S Q V S Q N Y T P D K K H Q K E P P F L W M G Y E L H
P D K W T V Q P I V L P E K D S W T V N D I Q K L V G K L N W A S Q I
Y A G I K V K Q L C K L L R G T K A L T E V I P L T E E A E L E L A E
N R E I L K E P V H G V Y Y D P S K D L I A E I Q K Q G Q G Q W T Y Q

IYQEPFKNLKTGKYARMRGAHTNDVKQLTEAVQKI
ATESIVIWGKTPKFKLPIQKETWEAWWTEYWQATW
IPEWEFVNTPPLVKLWYQLEKEPIVGAETFPISPI
ETVPVKLKPGMDGPKVKQWPLTEEKIKALVEICTE
MEKEGKISKIGPENPYNTPVFAIKKKDSTKWRKLV
DFRELNKRTQDFWEVQLGIPHPAGLKKKKSVTVLD
VGDAYFSVPLDKDFRKYTAFTIPSINNETPGIRYQ
YNVLPQGWKGSPAIFQSSMTKILEPFRKQNPDIVI
YQYMDDLYVGSDLEIGQHRTKIEELRQHLLRWGFT
TPDKKHQKEPPFLVWKFDSRLAFHHMARELHPEYY
KDCDPEKEVLVWKFDANEGENNSLLHPMSLHGMDD
PEKEVPEKVEEANEGENGPGIRYPLTFGWCFKLVP
VEPEKVEEWQNYTPGPGIRYQKRQDILDLWVYHTQ
GYFPDWQNYTPEGLIYSQKRQDIPMTYKAALDLSH
FLKEKGGLEGLIYSPQVPLRPMTYKAADCAWLEAQ
EEEEVGFPVRPQVPLRNTAANNADCAWLADGVGAV
SRDLEKHGAITSSNTAANNRRAEPAADGVGAMGGK
WSKRSVVGWPTVRERMRRAEPA

SEQ ID NO:10 Human immunodeficiency virus - Native NEF

MGGKWSKRSVVGWPTVRERMRRAEPAADGVGAVSRDLEKHGAI
TSSNTAANNADCAWLEAQEEEEVGFPVRPQVPLRPMTYKAALDL
SHFLKEKGGLEGLIYSQKRQDILDLWVYHTQGYFPDWQNYTPGP
GIRYPLTFGWCFKLVPVEPEKVEEANEGENNSLLHPMSLHGMDD
PEKEVLVWKFDSRLAFHHMARELHPEYYKDC

SEQ ID NO:11 Artificial sequence - gpn nucleotide sequence

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AACCTGCAAGGCCAGATGGTGCACCAGGCCATCAG
CCCCAGAACCCTGAATGCCTGGGTGAAGGTGGTGG
AGGAGAAGGCCTTCAGCCCTGAGGTGATCCCTATG
TTCAGCGCCCTGAGCGAGGGCGCCACCCCCCCAGGA
CCTGAACACCATGCTGAATACCGTGGGCGGCCATC
AGGCCGCCATGCAGATGCTGAAGGAGACCATCAAC
GAGGAGGCCGCCGAGTGGGACAGACTGCACCCCGT
GCACGCCGGACCTATCGCCCCTGGCCAGATGAGAG
AGCCCAGAGGCAGCGACATCGCCGGCACCACCAGC
ACCCTGCAAGAGCAGATCGGCTGGATGACCAACAA
CCCCCCTATCCCTGTGGGCGAGATCTACAAGAGAT
GGATCATCCTGGGCCTGAACAAGATCGTGAGAATG
TACAGCCCTACCAGCATCCTGGACATCAGACAGGG
ACCCAAGGAGCCCTTCAGAGACTACGTGGACAGGT
TCTACAAGACCCTGAGAGCCGAGCAGGCCAGCCAG
GAAGTGAAGAACTGGATGACCGAGACCCTGCTGGT
GCAGAACGCCAACCCCCGACTGCAAGACCATCCTGA
AGGCCCTGGGGACCTGCCGCTACCCCTGGAGGAGATG
ATGACCGCCTGCCAGGGCGTGGGCGGACCTGGCCA
CAAGGCCAGAGTGCTCATGGCCGCCAGAGCCAGCG
TGCTGAGCGGCGGAGAGCTGGATAGATGGGAGAAG
ATCAGACTGAGACCTGGCGGCAAGAAGAAGTACAA
GCTGAAGCACATCGTGTGGGCCTCTAGGGAGCTGG
AGAGATTCGCCGTGAATCCTGGCCTGCTGGAGACC
AGCGAGGGCTGTAGACAGATCCTGGGCCAGCTGCA
ACCCAGCCTGCAAACCGGCTCTGAGGAGCTGAGAT
CCCTGTACAACACCGTGGCCACCCTGTACTGCGTG
CACCAGAGAATCGAGGTGAAGGACACAAAGGAGGC
CCTGGAGAAGATCGAGGAGGAGCAGAACAAGTCCA
AGAAGAAGGCCCAGCAGGCCGCTGCCGACACCGGC
AACAGCAGCCAGGTGAGCCAGAACTACACCCCCGA
CAAGAAGCACCAGAAGGAGCCCCCTTTCCTGTGGA
TGGGCTACGAGCTGCACCCCGATAAGTGGACCGTG
CAGCCCATCGTGCTGCCTGAGAAGGATAGCTGGAC
CGTGAACGACATCCAGAAGCTGGTGGGCAAGCTGA

ACTGGGCCAGCCAGATCTACGCCGGCATCAAGGTG
AAGCAGCTGTGTAAGCTGCTGAGAGGCACCAAGGC
CCTCACAGAAGTGATCCCCCTCACAGAGGAGGCCG
AGCTGGAGCTGGCCGAGAACAGAGAGATCCTGAAA
GAGCCCGTGCACGGCGTGTACTACGACCCCAGCAA
GGATCTCATCGCCGAGATTCAGAAGCAGGGCCAGG
GCCAGTGGACCTACCAGATCTACCAGGAGCCTTTC
AAGAACCTGAAAACCGGCAAGTACGCCAGAATGAG
AGGCGCTCACACCAACGACGTGAAGCAGTTGACCG
AGGCCGTGCAGAAAATCGCCACCGAGAGCATCGTG
ATCTGGGGCAAGACCCCCAAGTTCAAGCTGCCTAT
CCAGAAGGAGACCTGGGAGGCCTGGTGGACCGAGT
ATTGGCAGGCCACCTGGATTCCTGAGTGGGAGTTC
GTGAACACCCCCCCTCTGGTGAAGCTGTGGTATCA
GCTGGAGAAGGAGCCTATCGTGGGCGCCGAGACCT
TCCCCATCAGCCCTATCGAGACCGTGCCCGTGAAG
CTGAAGCCCGGCATGGACGGCCCCAAGGTGAAACA
GTGGCCTCTCACCGAGGAGAAGATTAAGGCCCTGG
TGGAGATTTGCACCGAGATGGAGAAGGAAGGCAAG
ATCAGCAAGATCGGCCCCGAGAATCCCTACAATAC
CCCCGTGTTCGCCATTAAGAAGAAGGACTCCACCA
AGTGGAGAAAGCTGGTGGACTTCAGAGAGCTGAAC
AAGAGAACCCAGGACTTCTGGGAGGTGCAGCTGGG
CATCCCCCACCCTGCCGGCCTGAAGAAGAAGAAGA
GCGTGACCGTGCTGGACGTGGGCGACGCCTACTTC
AGCGTGCCCCTGGACAAGGACTTCAGAAAGTACAC
CGCCTTCACCATCCCCCAGCATCAACAACGAGACCC
CCGGCATCAGATACCAGTACAACGTGCTGCCCCAG
GGCTGGAAGGGCAGCCCCGCCATCTTCCAGAGCAG
CATGACAAAGATCCTGGAGCCCTTCCGGAAGCAGA
ACCCCGATATCGTGATCTACCAGTACATGGACGAC
CTGTACGTGGGCAGCGATCTGGAGATCGGCCAGCA
CAGAACCAAGATCGAAGAGCTGAGACAGCACCTGC
TGAGATGGGGCTTCACCACACCAGATAAGAAACAT
CAGAAAGAACCACCATTCCTGGTGTGGAAGTTTGA
CAGCAGACTGGCCTTCCACCATATGGCCAGAGAGC
TGCATCCTGAGTACTACAAGGACTGCGATCCCGAG
AAGGAGGTGCTGGTCTGGAAATTCGACGCCAACGA
GGGCGAGAACAACAGCCTGCTGCACCCCATGAGCC

TGCACGGCATGGATGATCCTGAGAAAGAAGTGCCC
GAGAAGGTGGAAGAGGCCAATGAGGGAGAAAACGG
CCCTGGCATTAGATATCCCCTCACCTTCGGCTGGT
GCTTCAAGCTGGTGCCTGTGGAGCCTGAGAAAGTG
GAGGAATGGCAGAATTACACACCCGGCCCAGGCAT
CCGGTATCAGAAGAGACAGGACATTCTGGACCTGT
GGGTGTACCACACCCAGGGCTACTTCCCCGACTGG
CAGAACTATACTCCTGAGGGCCTCATCTACAGCCA
GAAGCGGCAGGATATCCCCATGACCTACAAGGCCG
CCCTGGATCTGAGCCACTTTCTGAAAGAGAAGGGC
GGCCTGGAGGGCTTGATCTACTCCCCACAGGTGCC
ACTGAGACCTATGACATATAAGGCTGCCGACTGCG
CCTGGCTGGAGGCCCAGGAAGAGGAGGAAGTGGGC
TTCCCTGTGAGACCCCAGGTGCCCCCTGCGGAACAC
CGCCGCCAATAATGCCGATTGTGCTTGGCTCGCCG
ACGGCGTGGGAGCCGTGAGCAGaGACCTGGAAAAG
CACGGCGCCATCACCAGCAGCAATACAGCCGCCAA
CAACAGAAGAGCCGAGCCTGCCGCCGATGGAGTGG
GCGCCATGGGCGGCAAGTGGAGCAAGAGATCCGTG
GTGGCTGGCCCACCGTGAGAGAAAGAATGAGAAG
GGCCGAACCCCGCCAGAGGACCCGGCAGAGCCTTCG
TGACCATCTACCCCTACGACGTGCCCGACTACGCT
TGATGAGGCGCGCCCT

25

5-prime ApaI and 3-prime AscI sites are underlined. Initiating ATG and terminating TGA are in bold.

SEQ ID NO:12 Artificial sequence – disrupted pol

T P D K K H Q K E P P F L W M G Y E L H P D K W T V Q P I V L P E K D
S W T V N D I Q K L V G K L N W A S Q I Y A G I K V K Q L C K L L R G
T K A L T E V I P L T E E A E L E L A E N R E I L K E P V H G V Y Y D
P S K D L I A E I Q K Q G Q G Q W T Y Q I Y Q E P F K N L K T G K Y A
R M R G A H T N D V K Q L T E A V Q K I A T E S I V I W G K T P K F K
L P I Q K E T W E A W W T E Y W Q A T W I P E W E F V N T P P L V K L
W Y Q L E K E P I V G A E T F P I S P I E T V P V K L K P G M D G P K
V K Q W P L T E E K I K A L V E I C T E M E K E G K I S K I G P E N P
Y N T P V F A I K K K D S T K W R K L V D F R E L N K R T Q D F W E V
Q L G I P H P A G L K K K K S V T V L D V G D A Y F S V P L D K D F R
K Y T A F T I P S I N N E T P G I R Y Q Y N V L P Q G W K G S P A I F
Q S S M T K I L E P F R K Q N P D I V I Y Q Y M D D L Y V G S D L E I
G Q H R T K I E E L R Q H L L R W G F T T P D K K H Q K E P P F

SEQ ID NO:13 Artificial sequence – disrupted gag

M A P I V Q N L Q G Q M V H Q A I S P R T L N A W V K V V E E K A F S
P E V I P M F S A L S E G A T P Q D L N T M L N T V G G H Q A A M Q M
L K E T I N E E A A E W D R L H P V H A G P I A P G Q M R E P R G S D
I A G T T S T L Q E Q I G W M T N N P P I P V G E I Y K R W I I L G L
N K I V R M Y S P T S I L D I R Q G P K E P F R D Y V D R F Y K T L R
A E Q A S Q E V K N W M T E T L L V Q N A N P D C K T I L K A L G P A
A T L E E M M T A C Q G V G G P G H K A R V L M A A R A S V L S G G E
L D R W E K I R L R P G G K K K Y K L K H I V W A S R E L E R F A V N
P G L L E T S E G C R Q I L G Q L Q P S L Q T G S E E L R S L Y N T V
A T L Y C V H Q R I E V K D T K E A L E K I E E E Q N K S K K K A Q Q
A A A D T G N S S Q V S Q N Y

SEQ ID NO:14 Kozak sequence for Gag p24

GCC GCC ACC ATG G

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB2004/004038

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/62 C12N15/49 C07K14/16 A61K39/21

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 02/32943 A (CHADRABARTI BIMAL K ; HUANG YUE (US); US GOVERNMENT (US); NABEL GARY J) 25 April 2002 (2002-04-25) cited in the application the whole document page 4, line 1 - line 2 page 150 - page 154	1-3, 5-15,18, 19,21, 22,24, 25,27-48
A		4,16,17, 20,23,26
X	WO 01/47955 A (MEDICAL RES COUNCIL ; HANKE TOMAS (GB); MCMICHAEL ANDREW JAMES (GB); U) 5 July 2001 (2001-07-05) sequence 32	1-3, 5-15,18, 19,21, 22,24, 25,27-48

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 December 2004	27/12/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Galli, I

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB2004/004038

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KMIECIAK D ET AL: "Enhancement of cellular and humoral immune responses Human Immunodeficiency Virus Type 1 Gag and Pol by a G/P-92 fusion protein expressing highly immunogenic Gag p17/p24 and Pol p51 Antigens" JOURNAL OF HUMAN VIROLOGY, PHILADELPHIA, PA, US, vol. 4, no. 6, 2001, pages 306-316, XP008024317 ISSN: 1090-9508 the whole document	1-48

Form PCT/ISA/210 (continuation of second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2004/004038

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 35, 38, 41-46 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1-3, 5-6, 8-15, 18, 49
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 35, 38, 41-46 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box II.2

Claims Nos.: 1-3, 5-6, 8-15, 18, 49

Present claims 1-3, 5-15, 18, 19 cannot be searched over their whole breadth. Many of them are framed in terms of results to be achieved and without any truly technical characterization (see in particular claims 8, 14, 18). In many cases, the scope of protection is also ill-defined since it is framed in unclear, vague and broad parameters or disclaimed subject matter (claims 8-10 in particular). The claims thereby could be construed as referring to an unspecified and large amount of ill-defined compounds. It follows that there is an eminent lack of clarity in breach of Art. 6 PCT, lack of support under Article 6 PCT and lack of disclosure under Article 5 PCT, except for a very small number of the compounds claimed. A meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the GPN construct (seq. IDs 1, 9, 11).

Present claim 49 relates to an extremely large number of possible compounds. In fact, the claim covers so many options and undefined variables that a lack of clarity within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.